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BIOLOGICAL BULLETIN

REACTIONS OF AMEBA TO LIGHT AND THE EFFECT OF LIGHT ON FEEDING.

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INTRODUCTION.

The reactions of ameba-like organisms to light have been studied by a number of investigators in order to determine whether "naked" protoplasm is capable of responding to light waves. Whenever intense light was used as a source, amebas, pelomyxas and plasmodia reacted negatively, and as a consequence it was tacitly assumed that in general naked, "undifferentiated," protoplasm reacted negatively to light.

But speaking now only of experiments performed on amebas, for I do not wish to leave the impression that in my opinion what is true of the behavior of amebas toward light holds also for

plasmodia and pelomyxas, the first to be recorded appear to be those of Verworn ('89). He projected white light and various spectral colors of various intensities perpendicularly through the microscope slide, and observed no change of reaction as the ameba moved from one intensity to the other, or from one color to the other. With the experiment similarly staged, Davenport ('97) came to the same conclusions as Verworn. Amebas moved from a field of very weak light into one of very strong light, apparently without change of behavior, even when the change of intensity was sharp and abrupt. But Davenport showed that when a light beam is projected horizontally against an ameba, the ameba orients so as to flow away from the source of light. Harrington and Leaming ('00) showed that intense white, violet, or blue light, flashed on a moving ameba, arrested its movement momentarily; but red light was without any decisive effect. Mast ('10), experimenting under conditions similar to those under which Verworn and Davenport worked, confirmed Davenport's findings when horizontal beams of light are thrown against an ameba, and also concluded from his experiments that when an ameba finds a perpendicular beam of intense light in its path, it avoids the light in many cases. Mast also confirmed the general conclusions of Harrington and Leaming.

None of these investigators observed any but negative behavior, though Mast presumably looked for positive responses, for he says "I was unable to obtain positive reactions in *Stentor cæruleus*, *Amœba*, and fly larvæ" ('11, p. 270). The reason Mast failed to get positive responses was because the beams of light which he used were too large and the light was too intense. And further his apparatus was perhaps defective. He says: "The beam of light was produced by focusing a limited area of a luminous Welsbach mantle on the slide by means of *the mirror* and an Abbe condenser" ('11, p. 78). If he used an ordinary microscope mirror, as he seems to have, a very faint subsidiary beam as well as the very intense primary beam, was projected through the slide. The one is produced, of course, by the front surface of the glass, and is very readily overlooked; the other is produced by the surface of the silvering. Since the subsidiary image shows only on one or two sides of the main image, if square,

the chances are about even that the ameba came into contact with the subsidiary image first, and therefore the likelihood of a positive response is increased. From my own experience I have found that whenever mirrors are necessary, it is absolutely essential that they are silvered on the front surface; otherwise subsidiary images will result, and in case monochromatic spectral light is used a considerable degree of impurity may occur. If Mast used back surface mirrors in his work on the light reactions of ameba, some of his experimental results are, therefore, incompletely described and consequently inconclusive; and his inability to observe positive responses was due, in part, to improper staging of the experiment for this purpose.

The great majority of the light experiments in this paper show positive reactions; a few are indifferent, and a few negative. This proportion resulted from the manner in which the experiments were set, and from the fact that observations were based upon the behavior of amebas *before* they came into contact with the beam of light as seen by the eye, instead of after, as previous observers did. But in a great many cases negative behavior did not result even after the ameba came into contact with the light; but if the beams had been larger it is not improbable that the proportion of negative reactions would have been larger.

These experiments on the reactions of ameba to light were performed not only for the purpose of testing the sensitiveness of these organisms to light by itself, but especially to see whether differences in intensity, quality or direction of light rays are capable of causing changes in the behavior of amebas while feeding. In order to let the results speak as definitely as possible, a number of experiments were performed first with beams of light only as stimuli. Later, particles of food were presented in connection with the beams of light. By comparing these two sets of experiments with each other and with the results of previous experiments on the feeding habits of ameba (see Bibliography for references) the effect of light on feeding may be readily observed.

Several investigators, as already pointed out, made observations on the reactions of ameba to light, but all of them made use of large areas of very intense light. But for purposes of

comparison with other sources of stimuli it was thought essential, in my own work, to reduce the areas of light to a size comparable with the size of food and other objects which were placed at the disposal of the ameba. By throwing the beams of light vertically through the slide on which the amebas were placed, the area of the cross section of the beam of light used for stimulating the ameba could be varied within the desired limits.

As sources of light the Welsbach gas mantle and the Leitz Lilliput arc were used. The gas light was passed through five cm. of distilled water containing a very small quantity of an ammoniacal solution of copper sulphate to absorb the excess of green and yellow rays and the distinctive heat rays. Between the water and the microscope was interposed an opaque screen with several small, clear-cut pinholes in it. By moving the screen away from or toward the microscope, and focusing with the substage condenser, the size of the projected beams of light through the microscope could be easily controlled. The ordinary mirror of the microscope was discarded and a front surface mirror placed in its stead, in order to avoid reflection from the front surface of the glass and so producing a subsidiary image on the slide. The great sensitiveness of ameba to light makes this precaution absolutely necessary.

When spectral light was used, the arc was employed as a source, and either prism or grating interposed between clear distilled water and the screen.

The amebas were placed on large, clear thin cover glasses in clear culture fluid, without a cover glass over them. Usually in these light experiments the beams of light were left stationary while the coverglass containing the ameba was shifted, whenever shifting was necessary for experimental purposes. Both intermittent and continuous beams of light were employed. Intermittent light was produced by moving an opaque object up and down between the screen and the microscope. In a general way, continuous and intermittent light had about the same effect on the ameba. After orientation, it is worth noticing, the ameba advanced as definitely and as uniformly toward intermittent as toward continuous light.

The work was done in a dark room in which there was very

little diffuse light. Some light was necessary in order to make the camera lucida drawings illustrating the experiments. But this light was confined, as far as possible, to the paper on which the drawings were made, and no more light was used than was necessary. Extra precautions were taken to prevent any but vertical beams from reaching the ameba.

Experiments with Light.

White Light.—Two beams of white gas light were projected in front and to the right of an *Amœba dubia* flowing along in spatulate form—Fig. 1. The ameba flowed straight past the small spot of light and also passed the larger beam a short distance, when two side pseudopods were thrown out directly toward the larger beam. The pseudopod nearer the light spot enlarged until it had flowed over the light, then it was arrested and the other pseudopod became the main one through which the ameba moved away. The slide was then moved so that the small beam lay in front of the ameba—Fig. 5. Two pseudopods were formed on the left side. The main pseudopod moved into contact with the small beam of light, then sent out on the left a pseudopod which moved directly into contact with the larger beam. At the same time the more posterior of the previously formed pseudopods sent out on its right a pseudopod which also moved into contact with the larger beam while the more anterior pseudopod was withdrawn, but this pseudopod was finally withdrawn as the one which previously moved into contact with the light, moved on over the light spot—8. These experiments show very clearly that small beams of white light attract amebas before they actually come into contact with the beams.

An *Amœba proteus*[1] was placed so that a small beam of white light lay to the right of its path—10. Two pseudopods were thrown out, one on the left and one on the right, but both were quickly retracted. The tip of the ameba then turned sharply to the right and directly toward the light—14. The ameba then moved forward in the original direction for a short distance

[1] The name *Amœba proteus* of Pallas and Leidy as used in this paper also includes the species *A. discoides* Schaeffer, discovered to be distinct from *A. proteus* after the experimental work recorded in this paper was done (for preliminary descriptions see Schaeffer, '16*b*).

—16. Finally a pseudopod was thrown out on the right partly encircling the light—19. After breaking up into a number of pseudopods the ameba moved away. The ameba was shifted with the beam of light on the left—22. Movement became somewhat uncertain at first, then streaming was reversed—25. As the ameba moved forward in the new direction, it gradually turned to the right until it was flowing directly toward the beam of light—29. After partly surrounding the beam—35—the ameba moved away.

Summary.—From the foregoing experiments it is clear that ameba responds positively to white light under the given conditions. The ameba is attracted from a distance of sixty-five microns or more. Since the beam is projected vertically, the question at once arises: How does the ameba become aware of the beam of light? Is some light reflected horizontally by particles of solid matter in the water; or is some of the light energy transformed into heat or other form of energy which, being radially propagated, stimulates the ameba? These are very important questions in sense perception, but they must remain unanswered for the present. The light apparatus at my disposal was too crude to attempt a solution of them.

In most cases the ameba reacts positively until it comes into contact with the beam of light, when negative behavior usually sets in. This difference in behavior may be due to differences in the intensity of the stimulus. Both *proteus* and *dubia* react positively to white light.

It is quite clear from these experiments that light of the intensity used does not tend to inhibit directly the formation of pseudopods, nor does it seem to have any other direct effect on the movement or form of ameba.

Red Light.—The apparatus for producing monochromatic light of the various wave-lengths was a Leitz Lilliput arc light, fifteen cm. of distilled water, a piece of grating with a slit two millimeters wide, a screen of heavy drawing paper with a pinhole about one half mm. in diameter with clear-cut blackened sides, front surface mirror and condenser. The screen was blackened on the far side and lighted on the near side with just enough light from a Welsbach mantle to barely see the amebas. The spectrum on

the screen was about two cm. long. By means of the pinhole therefore only about one fortieth of its length was projected at any one time. The apparatus was rather crude and somewhat unsatisfactory, but it seemed to be sufficiently reliable for exploratory work.

On account of the impracticability of testing spectroscopically the beams of light which were thrown into the microscope, no accurate values in wave lengths can be given for blue, red, yellow, etc., so there may have been, for example, a few "rays" of orange or green in the yellow when that color is specified; but such mixtures cannot have been very significant since the size of the pencil of light was very small, roughly one fortieth of the length of the visible spectrum. As a matter of fact some rays of all wavelengths were mixed with the pencil of monochromatic light, but this defect could not be remedied, for some diffuse light (though this of course might be monochromatic spectral light) is necessary in order to see what is going on. But if any change in behavior takes place with reference to a pencil of monochromatic spectral light, the change must be caused by the more intense monochomatic light, since the diffused white light is equally intense all over the field.

A small beam of red spectral light was projected in the path of a *proteus*—37. The ameba moved forward a short distance, then sent out a pseudopod on the left—40. From this pseudopod another was sent out on the right—45—which moved directly toward and over the red beam. The pseudopod was soon withdrawn and the ameba then moved on.

Another *proteus* was brought into view with the light on the right—49. The ameba turned sharply to the right, then to the left, and finally moved over the red light. Presently a pseudopod was thrown out on the right through which the ameba moved away.

In the next experiment the light was placed on the left of a *proteus*—53. The ameba moved forward for a short distance, then threw out a pseudopod on the left—56—through which the ameba moved off without coming into contact with the light. The ameba was then shifted with the red beam lying on the left —59. As the ameba flowed forward toward the light spot a

pseudopod was thrown out on the right, through which the ameba finally moved off. The ameba was then shifted so that the light lay on the right—63. The ameba turned to the left and moved on. The ameba was again shifted with the light lying on the left—65. As the ameba moved forward a small pseudopod which was thrown out on the left moved directly into contact with the light. The next four experiments—69–93—all show that the beam of light was sensed before the ameba came into contact with it, and also that the light produced more or less definite positive behavior, followed by indifferent or negative behavior.

A beam of red light was projected in the path of another *proteus*—94. A pseudopod was thrown out toward the light. After it came into contact with the light—97—the ameba moved away through a pseudopod thrown out on the left—99. The ameba was then shifted with the beam of light on the left —101. The ameba moved forward, then turned slightly to the right—104. A small pseudopod was then sent out toward the red light—107—but after covering half the distance it was retracted while a pseudopod was thrown out on the same side, but further forward. From this pseudopod still another was sent out on the left. The ameba thus partly encircled the red light. The ameba was shifted again with the red light lying in front of it—111. The ameba threw out a pseudopod on the right and passed on—111–113—but when the tip of the main pseudopod extended beyond the light, it broke up into four pseudopods—115—of which the left posterior ultimately became the main pseudopod through which the ameba moved away.

Another *proteus* was then brought into the field with a beam of red light lying to the right—120. The ameba moved on a short distance, then threw out two pseudopods on the right—122 —one of which moved into contact directly with the red light —125—but which was retracted as the ameba moved on. The ameba was then shifted with the light lying on the left—125. Pseudopods were sent out on both sides but the one on the right finally became the main one leading the ameba away. The ameba was again shifted with the red light lying on the right—128. A pseudopod was thrown out on the right directly toward

the light. When it came into contact with the light it forked, the limbs moving forward with the light spot between them —133. The right limb became the main pseudopod through which the ameba moved off.

A beam of red light was projected to the right of another *proteus*—167. The tip of the ameba turned slightly toward the left, then resumed its original direction. The ameba was then shifted with the red light on the left—170. There was at first a tendency for pseudopods to form on the right, but, as the ameba moved forward, two were formed on the left in the region of the light. One of them moved a considerable distance toward the light—174—but was then retracted as a pseudopod on the right was thrown out to become eventually the main pseudopod. The ameba was then shifted with the red light lying on the left—176. As the ameba moved forward a large pseudopod was thrown out on the left, directly toward the light. This pseudopod became the main one through which the ameba moved away.

Summary.—Red spectral light produces about the same changes in behavior as white light. The vertical beam of red light is sensed at a distance, and in almost all cases produces positive behavior. In some few cases an ameba may behave indifferently or even negatively; but if the experiments are repeated several times a positive reaction is almost sure to occur. Amebas are therefore not negative or positive permanently with respect to beams of red light, but the behavior may readily change from the one to the other aspect. Red light of the intensity used does not seem to stimulate the ameba disagreeably when it moves into direct contact with the beam of light, for in a number of cases the ameba moved on over the light without visible change of behavior. In some respects the ameba tended to encircle the source of light in the same manner in which it sometimes encircles solid objects.

Blue Light.—A beam of impure[1] blue spectral light was projected to the right of a *proteus*—135. The ameba turned to the

[1] Between the grating and the arc was placed twenty cm. of distilled water containing a very little ammoniacal copper sulphate. The copper salt gave rise to a subsidiary faint yellowish spot of light not quite coinciding with the blue. The yellow image disappeared when the copper salt was omitted from the distilled water. The yellow image was probably due to fluorescence of the copper sulphate.

left and moved away, a decided negative reaction. The light was shifted so that it lay directly ahead of the ameba—139. The ameba threw out three pseudopods on the left, but almost immediately retracted them. Another was sent out on the right, but it also was soon retracted. The ameba then moved forward, and in passing the blue light sent out a little pseudopod toward it—143. The ameba finally turned to the left and moved on. The ameba was shifted again with the blue light on the right—145. After the tip of the ameba had passed by the blue light it turned to the right. A small pseudopod was also sent out toward the light—148. The ameba was then shifted with the blue light lying directly in front—150. As the ameba moved forward a small pseudopod was thrown out into contact with the light—152. Then the ameba moved on. The ameba was shifted again with the blue light directly ahead—154—but a decided negative reaction set in. But when shifted again with the blue light ahead—158—the tip of the ameba turned away from, and then toward the light, but finally moved on in the original direction. The ameba was shifted again with the blue light directly ahead—162. The resulting behavior was indefinite.

Another *proteus* was then brought into the field with a beam of blue light directly ahead—181. The ameba then threw out a pseudopod on the left and from this one another on the right, and from this last one still another on the right, so that the light was partially encircled. The ameba then moved off through a pseudopod on the left. The ameba was shifted with the blue light on the right—186. A pseudopod was thrown out on the right directly toward, and into contact with, the beam of light. The ameba then flowed away through another pseudopod on the right. The ameba was again shifted with the light lying on the right—190. Two pseudopods appeared on the right, one of which was directed toward the light spot. Both pseudopods were presently retracted and the ameba moved on to the left. Very peculiar behavior was observed when the ameba was shifted again—194. The behavior was at first negative, the ameba moving away to the right—195—but streaming was then reversed, and as the ameba passed by the light, two small pseudopods were sent out toward it—200. They were retracted however

as the ameba moved on. When shifted again with the blue light to the left—201—the ameba turned toward the light and then passed on to the left. The ameba again reacted positively when shifted with the light straight ahead—204. When shifted again the ameba reacted positively but rather uncertainly—209. In the next experiment, with the beam of blue light on the left, negative behavior was induced—216.

A beam of pure (see footnote p. 11) blue spectral light was projected to the right of a *dubia*—219. The ameba moved past the light spot for a considerable distance without any change in behavior. Then two pseudopods were sent out: one directly toward the light, and the other near the tip, but also on the right side—220. As the pseudopods enlarged, the tip of the ameba also turned sharply to the right—221. When the posterior pseudopod came into contact with the light, the pseudopods on the right were retracted, and two others thrown out on the left —222—but these also were retracted after a few seconds, and the ameba then moved on in the original direction. The ameba was then shifted with the blue light lying directly ahead—223. The tip of the ameba (only the tip of the ameba is shown) turned to the left—224—but a pseudopod was thrown out on the right toward and into contact with the light—225. The ameba flowed partly over the light—226—but withdrew from it later and moved off through a pseudopod on the right.

Summary.—There is no marked difference between the reactions toward red light and those toward blue. Blue light induces positive behavior in as marked a degree as red, though when all the experiments are considered, red light seems to be somewhat more attractive than blue. Blue light, like red and white, induces both negative and positive reactions. Blue light can also be sensed at a distance.

The experiments with the *dubia*—219–222—are interesting inasmuch as a pseudopod was thrown out at the tip of the ameba on the side on which the light lay, some time after this part of the ameba had passed the light. It may be noted also that the tip of the ameba turned strongly in the same direction. It appears quite unlikely that the light acted as an efficient cause on this region of the ameba at the time of the formation of the pseudopod,

for the effect of the light was continually decreasing in intensity as the ameba moved away from it. The light would therefore be expected to have the maximum effect at maximum intensity, which was when the tip of the ameba was closest to the light. It is improbable therefore that the throwing out of a pseudopod and the bending of the tip to the right were caused by the impinging of the light rays at that region at that time. It is possible that this behavior is the result of the cumulative effect of the light rays while the ameba was passing the beam. There was formed a tendency toward a positive reaction some few seconds before it expressed itself in visible change of behavior, and when this tendency "came to a head" it resulted in exaggerated behavior; for two pseudopods were thrown out on the stimulated side and at the same time the tip of the ameba was turned to the right. This feature of ameban behavior—the formation of two pseudopods on the stimulated side, one near the anterior end and the other opposite the stimulating object after the tip of the ameba has passed the stimulating object—is frequently observed and is of great interest. It indicates several things. First, it effectively disposes of the hypothesis that the movement of pseudopods toward an object is directly induced by the object. Second, it shows that there is some sort of a coördinating or integrating agency at work in the ameba so that the larger part of it, at least, tends to react in a coördinated manner, even if there are separate centers of reaction. When the posterior pseudopod came into contact with the light, negative behavior set in suddenly. The pseudopods on the right were promptly withdrawn and two others were rapidly projected on the left. Nevertheless, the ameba finally moved on in the original direction.

Violet Light.—The violet light that was selected was as near to the end of the visible spectrum as possible. A beam of violet light was projected on the right of a *proteus*—230. A small pseudopod was thrown out on either side—232—the one on the right being directed toward the light. The ameba moved away however through the pseudopod on the left. The ameba was shifted with the light again on the right—235. The tip of the main pseudopod turned to the right and moved into contact with and then on over the light. The ameba was shifted with the

violet light on the left—241. As the ameba moved forward past the light a small pseudopod was sent out toward the light, but it was withdrawn before it came into contact with the light.

When shifted again with the light on the right—245—the ameba sent out a pseudopod anterior to the light—249—but it curved backwards toward the light as the ameba moved forward—251. On the next trial—253—the ameba first turned away from the light then sent out a pseudopod directly into contact with it; then another pseudopod was sent out on the side and anterior to this one.

Summary.—Amebas react positively, negatively, or indifferently toward violet light. The greater number of changes of behavior produced by violet light were positive. No definite differences could be observed between the effects of violet light and those of any other spectral light thus far described.

Green Light.—A beam of green spectral light was projected to the right of an *Amœba dubia*—259. As the ameba moved forward the tip of the main pseudopod moved to the left. A small pseudopod was formed on the right toward the light. The ameba then turned toward the right and at the same time threw out a pseudopod on the right near the tip of the main pseudopod. Both pseudopods were withdrawn as the ameba moved on. (Compare the behavior of this ameba with that illustrated in Figs. 219–222.)

The beam of green light was then projected to the right of a *proteus*—262–265. As the ameba moved forward, a large pseudopod which was thrown out on the left, was soon retracted, the ameba moving on in a straight path. The ameba was shifted—266–270—with the green light to the right. As the ameba moved on past the light, a small pseudopod appeared on the right near the light, but it was retracted before it had developed to any extent, as the ameba flowed on.

These few experiments indicate that the effect of green spectral light is similar in a general way to that of white, red, blue, etc. Although the positive reactions in these experiments are slight, they are nevertheless definitely positive. If I had made as many experiments with green light as with red or blue, I have no doubt that more decided reactions would have been obtained.

Yellow Light.—A beam of yellow spectral light was projected straight ahead of a *proteus*—271. The ameba moved on without any definite change of behavior and passed over the beam of light. The change in the direction of movement—275–277—indicates that the yellow beam had a disagreeable effect after the ameba came into contact with it. When the ameba was shifted—278—the tip of the main pseudopod turned to the left—a negative reaction continued from the previous experiment. But while passing the beam of light the negative condition gave way to a positive as is shown by the turning of the tip of the ameba toward the light—282. A pseudopod was then thrown out on the left on the convex side, and from this one another on the left through which the ameba moved on, again a negative reaction. The ameba was shifted again—285—with the yellow light ahead. The ameba turned sharply to the right, but as it passed by the light a pseudopod was thrown out on the left directly toward the light—287. This pseudopod became the main one through which the ameba flowed on over the light. As the ameba came nearly into contact with the light, a pseudopod was thrown out on the right—290—an indication of a negative reaction, but it was soon retracted.

To summarize: Amebas respond positively, negatively or indifferently to beams of yellow spectral light. As far as my experiments go, yellow light has about the same effect as red or blue or the other spectral colors which have so far been considered.

Orange Light.—A beam of orange light was projected to the left of a *proteus*—294. The ameba turned to the left and moved directly into contact with the light. When the ameba came into contact with the light, a pseudopod was started on the right, but it was soon retracted and the ameba flowed on over the light without further change of behavior. The ameba was then shifted with the orange light on the left—301. The tip of the ameba turned to the left, then broke up into two pseudopods of which the left one turned still further to the left and finally became the main pseudopod through which the ameba flowed away. The ameba was shifted again with the orange light slightly to the right—306. A pseudopod which was thrown out on the right elongated as it turned to the left. When the tip of the pseudo-

pod had passed the light, a new pseudopod was thrown out on the left near the light—309. This pseudopod moved straight forward for some distance, when another pseudopod was sent out on the left—312—but this one was finally retracted as the ameba moved away.

Orange spectral light induced positive reactions in the ameba of this series of experiments, though they were wholly positive only in the first experiment. In the other experiments the tendency was toward positive behavior, but the source of stimulation was not definitely sought. The beam of light attracted the ameba only mildly after the first encounter, and the tendency to move forward (Schaeffer, '14*a*) may be presumed to have been about as strong as the tendency to move toward the beam, hence the partial encircling of the beam in the last two experiments. In my laboratory notes there is recorded one experiment with orange light in which the behavior was wholly negative.

Experiments with Dark Beams.

When it was seen that white light and spectral light of various wave-lengths had essentially the same effect on ameba, it seemed likely that these results were due to differences in intensity between the beam of light and the diffuse light on the field. The suggestion then presented itself whether a decrease in intensity of light in a small area produces a similar result. A dark beam was therefore projected into the microscope. The source of the dark beam was a hole in the screen, leading into a blackened light tight box fastened to the back of the screen. The sides of the hole were blackened to prevent as far as possible the reflection of light. The rest of the screen was illuminated by diffuse light, as in the other experiments, but more brightly so as to increase the contrast between the field and the hole. The hole as viewed through the microscope appeared as a very dark gray spot.

A *proteus* was shifted so that the dark spot lay directly ahead of the ameba—314. The tip of the ameba broke up into two pseudopods, one of which turned to the right and the other to the left of the dark spot, indicating a negative reaction. As the right pseudopod moved forward it turned to the left until it

came into contact with the dark spot—a positive reaction. The ameba then moved on through this pseudopod and partly over the dark spot. The ameba was again shifted so that the dark beam lay directly ahead—319. A pseudopod which was thrown out toward the right led the ameba away, a definite negative reaction.

Another *proteus* was then brought into the field with the dark spot directly ahead—322. The ameba turned to the right and moved on, avoiding the dark beam. The ameba was then shifted with the dark spot straight ahead—325. The ameba became irregular in its streaming at the anterior end, indicating that the ameba sensed the dark spot and that there was present a tendency to react negatively; but the tendency to negative reaction was weak, for the ameba started presently to move over the dark area. The ameba, in irregular shape, was shifted again with the dark spot directly ahead—329. When the ameba came into contact with the outer edge, the tip of the main pseudopod forked, the right prong becoming the main pseudopod through which the ameba moved away. Negative behavior is again shown here. The ameba was shifted with the dark spot directly ahead—333. A pseudopod was thrown out on the left as the ameba moved into contact with the dark spot—334—indicating a tendency to negative reaction, but it was withdrawn as the tip of the ameba proceeded for some distance beyond the further edge of the dark area—336. The ameba was moved again so that the dark spot lay slightly to the right—340. The ameba moved into contact with the dark area, then sent out a pseudopod on the left, but it was soon withdrawn and at the same time another was sent out on the right. The ameba finally moved on in the original direction. Here we have first positive behavior in the turning of the ameba toward the dark spot; then negative behavior in the formation of the pseudopod on the left; then again positive behavior in the resumption of forward movement and the formation of the pseudopod on the right. In the next trial the ameba was moved with the dark spot slightly to the right—346. The ameba turned slightly further to the left, then directly toward the right and toward the dark spot—348. When the ameba came into contact with the dark spot, a pseudopod was thrown out on the left, but it was withdrawn as the ameba moved on.

Another *proteus* was then brought into the field with the dark spot slightly to the left—354. The tip of the main pseudopod broke up into two pseudopods of which one moved directly toward and over the dark area. But when the ameba came into contact with the dark spot, a large pseudopod was thrown out on the right—356—but it was withdrawn as the ameba moved on over the dark spot. The ameba reacted in effect positively throughout the experiment, but a strong tendency to react negatively is shown by the breaking up of the main pseudopod into two pseudopods—355—and by the appearance of the pseudopod on the right when the ameba came into contact with the dark spot —358. The ameba was then shifted with the dark spot slightly to the left—359. The ameba moved forward a short distance, then the tip of the main pseudopod spread out, and then the protoplasmic stream was suddenly reversed and the ameba moved away to the right through a vestige of a previous pseudopod—a decided negative reaction. But the ameba was then moved with the dark spot directly ahead—363. After the ameba had moved forward a short distance the tip forked broadly, and the ameba moved off through the left prong, again a decided negative reaction.

Summary.—Amebas become aware of dark spots before they come into contact with them, as seen through the microscope with the eye, just as they become aware of beams of light before encountering them. In most cases the tendency is to react negatively, but in some instances the first change in behavior is positive. Usually when the ameba first comes into contact with the dark beam there is a tendency toward negative behavior, as is shown by the formation of pseudopods which, if they became main pseudopods, would lead away from the dark area. These pseudopods are usually withdrawn as the ameba moves forward over the dark spot. The behavior is seldom wholly positive or wholly negative; in most cases there is some vacillation between negative and positive reactions. The reactions on the whole were not so pronounced as those toward light. The actual stimulating quality is very likely to be looked for in the difference in light intensity between the dark spot and the field.

REACTIONS TOWARD SOLID PARTICLES WHEN STIMULATED AT THE SAME TIME BY BEAMS OF LIGHT OR OF DARKNESS.

A grain of globulin was placed over a small beam of blue spectral light, and arranged so that the illuminated globulin lay in the path of an *Amœba proteus*—365. The ameba moved in spatulate form directly toward the globulin-blue light until it came into contact with the globulin, when a pseudopod appeared on the right. The ameba however ingested the globulin in typical manner and then quieted down over the blue light for over twelve minutes.

A grain of globulin was placed in the path of a *proteus* with a beam of green spectral light between the ameba and the globulin —373. Through a pseudopod thrown out on the right the ameba moved away from the light-globulin—374. The ameba then broke up into four pseudopods of which the left one of the middle pair became the main pseudopod. The ameba moved forward through this pseudopod toward the globulin in a curved path, apparently, so as to avoid the light, pushed the globulin ahead a short distance, and then ingested it in an imperfect food cup. This is an interesting experiment. The beam of green light stimulated the ameba negatively when contrasted with the globulin. The ameba made a detour around the light to get to the globulin. This experiment should be compared with Figs. 1–13 in a recent paper (Schaeffer, '17*b*) in which very similar behavior is recorded as an ameba moved toward a grain of globulin with a grain of silicic acid lying immediately in front of the globulin.

A grain of globulin was placed to the left of a *proteus* with a beam of green spectral light between the globulin and the ameba —386. The ameba moved forward a short distance, then bifurcated, the right prong being directed backwards while the left prong was directed toward the globulin—388. The ameba moved toward the light at first—389—but presently the tip of the ameba broke up into two pseudopods. The one thrown out on the right enlarged rapidly as it moved in a slight detour around the light toward the globulin—392, 393. After rolling the globulin along the surface for a short distance it was ingested in a typical food cup. This experiment as well as the preceding,

shows that a beam of green light acts as a disturbing factor when an ameba is stimulated at the same time by globulin.

A grain of globulin and a beam of yellow spectral light were placed to the right of the path of a *proteus* with the light between the globulin and the ameba—397. As the ameba moved forward it turned to the right and directly toward the yellow light. The ameba moved over the light, then turned to the left and moved into contact with the globulin, which the ameba rolled around a short distance before ingesting it in a normal food cup. The yellow light did not disturb the ameba when stimulated simultaneously by globulin.

A grain of globulin and a beam of yellow light arranged as in the preceding experiment were placed in the path of another *proteus* —408—but the behavior observed was negative, due doubtless to lack of hunger in the ameba. The ameba was in Y-shape at the beginning of the experiment—408. The ameba responded negatively by bending the right prong to the right and flowing along it. A pod was thrown out on the right, indicating the presence of a tendency to a positive reaction. The ameba was then shifted with the yellow beam straight ahead and the globulin a little to the left—412. The tip of the ameba forked, the axes of the limbs coinciding with the same straight line, and nearly perpendicular to the rest of the ameba—413. The right prong turned toward the yellow light—415—and presently two pseudopods were sent out a short distance toward the globulin—416 —but both were withdrawn as the ameba moved away through a pseudopod thrown out on the right—417. The ameba was shifted with the globulin straight ahead and the beam of yellow light to the left—419. The ameba moved forward a short distance when a pseudopod was thrown out to the left—421. This pseudopod became the main one, and after it flowed ahead some distance it turned to the right and moved toward the globulin —423. The tip of the ameba then turned to the right still more strongly and at the same time a pseudopod was thrown out in the direction of the stimulating objects—424. The posterior end now became activated but only for a short time—425. Several new pseudopods were formed indicating uncertainty in behavior, of which the one on the right extending toward the globulin

became for the moment the main pseudopod—427. After moving toward the globulin for a short distance a pseudopod was thrown out on the right leading the ameba away from the test objects—429-432. Presently however the main pseudopod bent strongly to the left—433, 434—with the formation at the same time of two pseudopods on the convex side of the main pseudopod—434, 435. The pseudopod pointing toward the globulin moved forward a short distance—436—but this pseudopod was retracted as the other one of the two formed on the convex side became reactivated leading the ameba away from the globulin and light.

Since it was evident that the ameba reacted negatively to both light and globulin when these substances stimulated the ameba at the same time, two further tests were made on this ameba in which each test substance was used by itself.

The piece of globulin was laid some distance ahead of the ameba —438. The ameba moved toward the globulin a short distance then threw out a pseudopod on the left—440—through which the ameba moved forward with the globulin on the right—442. A pseudopod was then thrown out on the right toward the globulin while the tip of the main pseudopod turned strongly toward the left—444. When the pseudopod on the right came into contact with the globulin the main pseudopod was retracted —447, 448. The globulin was only partly surrounded—449. One of several pseudopods formed on the right led the ameba away, leaving the globulin behind.

After a few minutes the beam of yellow light was projected in front of this ameba—453. As the ameba moved forward several pseudopods were thrown out on either side of the main pseudopod, giving the ameba a very irregular shape. When the ameba came nearer the light—458—it advanced definitely forward passing the beam immediately to the right—459. The main pseudopod then swerved a little to the side as two pseudopods were formed on the right, through the lower one of which the ameba finally moved away from the light.

This ameba then reacted definitely positively to globulin but much less definitely positively to yellow light, when presented separately, but decidedly negatively when presented together.

The experiment shows that yellow light was the cause of the negative behavior when both it and globulin were presented together; or perhaps one ought to say yellow light, *in combination*, possessed deterring qualities which were absent when it stimulated the ameba alone. Here again we have another case of where the milder of two positively stimulating objects when encountered alone becomes negative when encountered simultaneously with the more strongly stimulating object (Schaeffer, '17*b*).

A dark spot with a piece of carbon lying in it was then placed in the path of a *proteus*—463. As the ameba moved forward it turned slightly to the left at first—467—but the tip of the ameba then turned to the right and moved directly toward the dark beam and carbon—471—until within about ten microns of the beam, when the ameba suddenly turned to the right—472. The ameba moved along in this direction without coming into contact with either the dark spot or the carbon. That this decided negative reaction was caused by the dark spot is evidenced by the next experiment in which the carbon was omitted—481. The ameba moved forward toward the dark beam for a short distance, then became very uncertain in its behavior. First it turned to the right, then formed a pseudopod on the left through which it moved forward with the dark spot on the right—484. A pseudopod was thrown out on the left but it was promptly retracted—487. At the same time the tip of the pseudopod turned very sharply to the right and moved directly toward the dark spot—488. The ameba continued moving toward the dark spot until within thirty microns of it—489—when the main pseudopod was retracted and the ameba then moved away to the left through another pseudopod which had been slowly forming while the ameba was moving toward the dark beam. A dark beam with a piece of globulin lying in its centre was placed to the right of the ameba with a pseudopod already turned toward the dark spot—492. The ameba flowed on through the pseudopod directly toward the dark spot. When within about eighty microns of it, the tip of the main pseudopod forked—495—indicating a tendency to negative behavior. The left prong which became the main pseudopod, moved directly

forward until it came nearly into contact with the dark beam when a slender pseudopod moved into the dark area, and another, indicating a tendency to negative behavior, moved to the right —498. But when the ameba came into contact with the globulin the pseudopod on the right was withdrawn and then the globulin was ingested, the ameba quieting down over the dark spot. The experiments with this ameba show that the dark beam may be sensed at a distance of at least 150 microns, and that the globulin may be sensed as well apparently when lying on a dark spot as when illuminated.

A dark spot with a fragment of carbon lying on it was placed to the right of an ameba—503. There was no definite response. The ameba turned toward the right and moved toward the test objects in a more or less uncertain manner—504, 505. A pseudopod was then sent out on the left—506. After it had attained to considerable size it was withdrawn and the ameba moved off through a pseudopod thrown out on the right—508. The ameba was shifted with the carbon-dark spot lying on the right—509. As the ameba moved forward it turned toward the right—510—a small pseudopod being formed, as frequently happens, on the convex side, but a pseudopod was presently thrown out on the left—513—through which the ameba moved away. To show that it was the dark spot and not the carbon which produced the negative behavior, the results of the next experiment are appended—514. The ameba moved directly into contact with and on over the carbon without any sign of a negative reaction. A grain of globulin lying in a dark beam was then placed at some distance to the right of the ameba—520. An accidental jar displaced the globulin so that it lay near the anterior edge of the dark spot. The ameba changed its direction of motion and moved straight toward the globulin and the dark beam—521. (It may reasonably be doubted whether the ameba sensed the globulin at this distance.) The globulin was then moved to the further side of the dark beam—522. The ameba kept on moving forward for some distance, then its behavior became somewhat uncertain. The ameba moved slightly to the left—524. A pseudopod which was thrown out on the right—525—became later the main pseudopod. The ameba now moved directly

toward the dark spot until within about twenty microns—528—when the protoplasmic current was reversed and the ameba moved off through a pseudopod on the left near the posterior end—529. Soon this pseudopod was withdrawn and the ameba flowed into another pseudopod (perhaps the vestige of the former main pseudopod) which led the ameba to the right (to the left of the dark spot)—533. After moving some distance in this direction, a pseudopod was thrown out on the left directly opposite to the dark spot—534. Two more pseudopods were then formed on the right through the more posterior of which the ameba moved on —535–537. A pseudopod was then formed on the left which led the ameba out of range of the dark spot—538. The ameba was then shifted with the dark spot on the right and the grain of globulin just in front of it—540. The ameba turned to the left—541—and sent out on the convex side a pseudopod through which the ameba moved forward with the dark spot on the left—543. As the ameba moved on it turned toward the dark beam but presently two pseudopods were thrown out from the middle of the ameba, one on either side—546. (The pseudopod on the left was evidently formed to enable the ameba to move at once into contact with the globulin; the one on the right was formed without assignable cause, although opposite pseudopods are frequently formed under conditions similar to these.) The pseudopod on the left moved through a curved path to the left into contact with the globulin—547–550. When the ameba came into contact with the globulin, it was pushed into the dark area. A slender pseudopod followed it while the tip of the main one remained stationary for the moment, which indicates that the dark area had a deterrent effect on the ameba—551. A food cup was however soon formed and the globulin ingested.

Conclusions.

From these experiments it may be concluded that white light and all the visible spectral colors cause positive responses; but whether all are equally attractive cannot be definitely stated, for experiments would have to be staged differently to produce accurate results. Nevertheless the red end of the spectrum seems to be somewhat more attractive than the blue.

What is of considerable interest in the behavior of ameba toward light is that the character of the response may vary rapidly. See Figs. 22 to 36; 37 to 48; and a number of other experiments. A negative reaction may be followed by a positive and vice versa. There is no definite relationship between ameba and light, on account of which the ameba is always either positive or negative or indifferent. Stimulation from light produces the same general character of reaction as stimulation from glass or carbon. The only observable difference is a quantitative one; light beams are sensed at a greater distance than particles of glass or carbon. This difference may however be due to a difference in intensity of the stimuli.

Ameba reacts to dark spots in much the same way that it does to beams of light. The reactions are either positive, negative or indifferent. But they are negative in much the greater number of cases. But no sooner does one observe the reactions of an ameba to perpendicular beams of light and of darkness than the question arises as to the transfer of the stimulus to the ameba as well as the nature of it. How can an ameba sense a beam of light or darkness which never comes nearer to it than 100 or 150 microns? It is possible that small particles suspended in the water reflect light from a beam of light so as to reach the ameba in much the same way that man can observe a beam of light in a dark room because of the dust particles in the air. But if so the ameba, being eyeless, is wonderfully sensitive to light. But as to beams of darkness the case is entirely different. Is it conceivable that an ameba can sense a beam of darkness at a distance because not as much light is reflected from the particles in the dark beam as from those more brightly illuminated surrounding the beam? If one did not know of reactions to beams of darkness, one might adopt the hypothesis of the reflection of light from particles in the beam; but since similar behavior is observed toward beams of darkness, this explanation is obviously not the right one. Some disturbance is created by the beams which is then radially transmitted; so much is certain. But just what is the nature of the disturbance is not clear.

In a preceding paper (Schaeffer, '16*c*), in which the reactions of ameba to particles of glass, carbon, and similar materials were

described, it was concluded that the nature of the stimulus which enabled amebas to react to these substances at a distance also remains unrecognized. Now it is possible that the nature of the stimulus which makes reaction at a distance possible is the same for all these various test objects, since the reactions are very similar. If so, the nature of the stimulation must be simple and fundamental, such as differences in electrical potential which give rise to electrical currents. But if the nature of the stimulation should be electrical, the quantities of current arising from the various test objects must be infinitesimally small, and very great if not insurmountable difficulties would be encountered in demonstrating the presence of such small currents.

To show the general reactions of ameba to globulin, carbon, etc., when stimulated simultaneously by beams of light or of darkness, the experiments may be classified as follows.

1. Food objects (grains of globulin) were laid *over* a beam of intense light so that the food should be very brightly illuminated —365–372. Blue spectral light was used in the experiments recorded, for blue light has been regarded as more disagreeable than other spectral colors. The globulin was sensed at a distance and the ameba moved toward it and ingested it. There was no definite indication that the blue light had any effect in modifying the behavior unless the pseudopod to the right in Fig. 368 is to be regarded as expressing a deterrent effect of the light. The ameba, in effect, reacted as if no spectral blue light was present.

2. The food substance was laid *some distance from* the green or yellow light, and in various positions with respect to the ameba and the beam of light—373–437.

(*a*) When the green light lay between the ameba and the globulin, the light had a slight disturbing and deterring effect —386. The ameba made a slight detour around the green light. In another test with the experiment similarly staged, the disturbing effect of the green light was more pronounced—373. The ameba made a wide detour around the light and moved into contact with the globulin without coming into contact with the green beam. In both experiments green light, which is positive when sensed alone, became negative in contrast with the more strongly (or differently) positive globulin.

(*b*) In the experiment with yellow light—397–407—the ameba moved straight toward the light after the globulin was within sensing range, then moved over the beam of light, after which the direction of motion was changed so that the ameba moved directly toward the globulin. The globulin was eaten in a typical food cup. The yellow light was not deterrent in this case. But another ameba reacted negatively to both yellow light and globulin, when presented simultaneously, but positively when presented separately. The ameba was satiated or sick, for the globulin was only partially surrounded.

3. Grains of globulin and carbon were laid over beams of darkness.

(*a*) An ameba moved toward a dark spot on which lay a grain of carbon until it came within about thirty microns of the dark spot, when negative behavior set in. The ameba moved away to the right—463. In the succeeding test the ameba reacted at first positively to the dark spot alone, and after that decidedly negatively.

(*b*) A piece of globulin was laid on the dark spot, to the right of the ameba. The ameba moved directly toward the dark spot —globulin—though it seemed to have been slightly deterred by the dark area, for the ameba broke up into two pseudopods—495 —and just when the dark beam was reached a little later, a small side pseudopod appeared. The globulin was however finally ingested. In another experiment the globulin was placed near the far edge of the dark spot—522. The behavior of the ameba became very irregular as it moved near the dark beam. Soon a pseudopod was sent out straight toward the globulin, but it was presently retracted and the ameba moved off to the left, veering to the right. There can be no doubt of the strongly deterrent effect of the dark beams. There can also be no doubt of the strongly attractive effect of the globulin.

Summary.

1. Ameba senses beams of light of twenty microns' diameter that pass no nearer to the ameba than 100 microns or 150 microns. In nearly all cases under these conditions the ameba moves directly toward the beam. When the ameba comes into contact

with the beam it either flows over it indifferently, or it reacts negatively to the beam by changing its direction of movement.

2. Beams of spectral light and of white light have approximately the same general effect. It appears however that spectral light at the blue end is somewhat less attractive than that at the red end.

3. Beams of darkness are also sensed at a distance like beams of light. They are usually negative. That is, the ameba usually avoids the beams before coming very near them.

4. It is the change of light intensity that determines changes in reactions. Neither high nor low intensities seem to be either negative or positive in themselves. Movement from a region of low light intensity into a region of high intensity frequently occurs if the contrast is not too great; but movement toward a region of lower light intensity (dark beams) is seldom seen.

5. No explanation is suggested for the sensing of beams of light and of darkness at a distance. The nature of the stimulus and the means of its transfer in such cases is not known.

6. Grains of globulin illuminated by perpendicular beams of light seem, on the whole, to be at least as attractive as when not more brightly illuminated than the field. But when globulin grains are laid in large dark beams, the ameba frequently shows unmistakable signs of a tendency to react negatively.

7. Both light beams and globulin grains are positive when stimulating the ameba separately; but when a grain of globulin and a beam of light, placed a small distance apart from each other, stimulate the ameba simultaneously, the more weakly positive object—the beam of light—becomes usually strikingly negative.

8. An ameba is positive, negative or indifferent to beams of light depending upon circumstances.

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EXPLANATION OF PLATES.

The figures are camera lucida drawings of sample experiments taken from the laboratory notes without alterations. The camera lucida was attached to the right-hand tube of a long-arm Zeiss binocular microscope, which was used in connection with the stage and condenser of a compound microscope. Eyepieces number 4 and objective a₃ were used, giving a magnification of 65 diameters. A scale by means of which the size of amebas and light beams can be estimated is shown on Plate V.

The figures are numbered serially from 1 on, for reference. The numbers are placed inside the figures. They are to be looked upon as labels only. They have no other significance. An x following a number, as 21x, indicates the end of the experiment illustrated by Figs. 10 to 21x inclusive. A new experiment starts with Fig. 22 and ends with Fig. 36xx, and so on. If a number is followed by xx, it means that the next experiment was performed upon a different ameba. Thus Figs. 1 to 9xx represent the result of a single experiment upon an ameba. With Fig. 10 a new ameba was employed, and so on. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed on any one ameba.

The time of the beginning and the end of each experiment is given in hours and minutes. In some cases the time of drawing of each figure is also given, and where it is not given it may easily be computed—the figures in such case being spaced equally in time.

The arrows show the direction of active protoplasmic streaming. The larger arrow in the last figure of each experiment denotes the direction the ameba took in moving away from the test object.

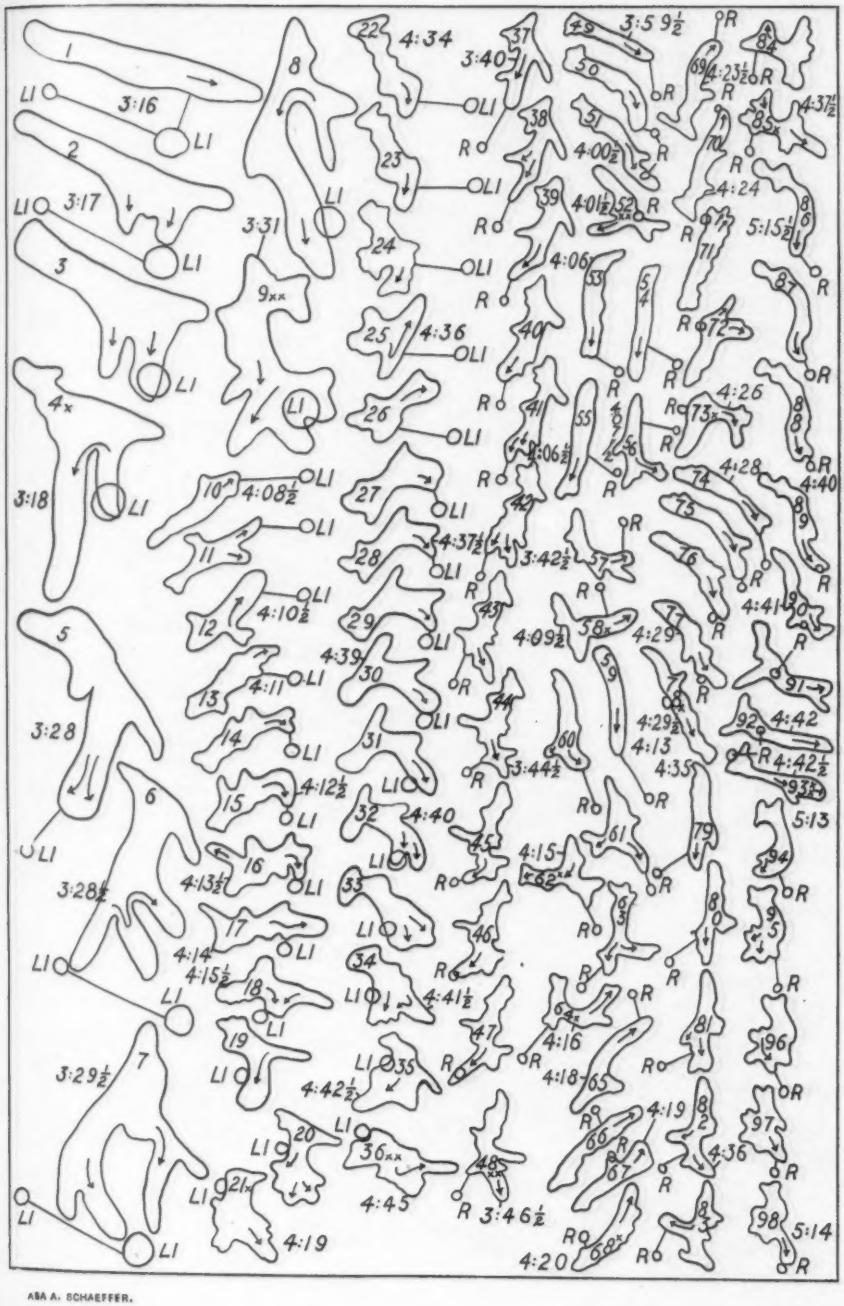
The light beams, etc., are labelled in abbreviated form. See table of abbreviations on below. For quick and correct reference these test objects are connected with the proper ameba by leader lines. These lines have no other significance.

It will be noted that there are slight differences in the size and shape of the same light beam or other test object as drawn in the figures of any single experiment. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

For detailed explanation of figures see pages 49–67 of the text.

Table of Abbreviations.

B, blue light.	*LI*, white light.
CA, carbon.	*OL*, orange light.
D, dark beams.	*R*, red light.
G, globulin.	*V*, violet light.
GN, green light.	*Y*, yellow light.



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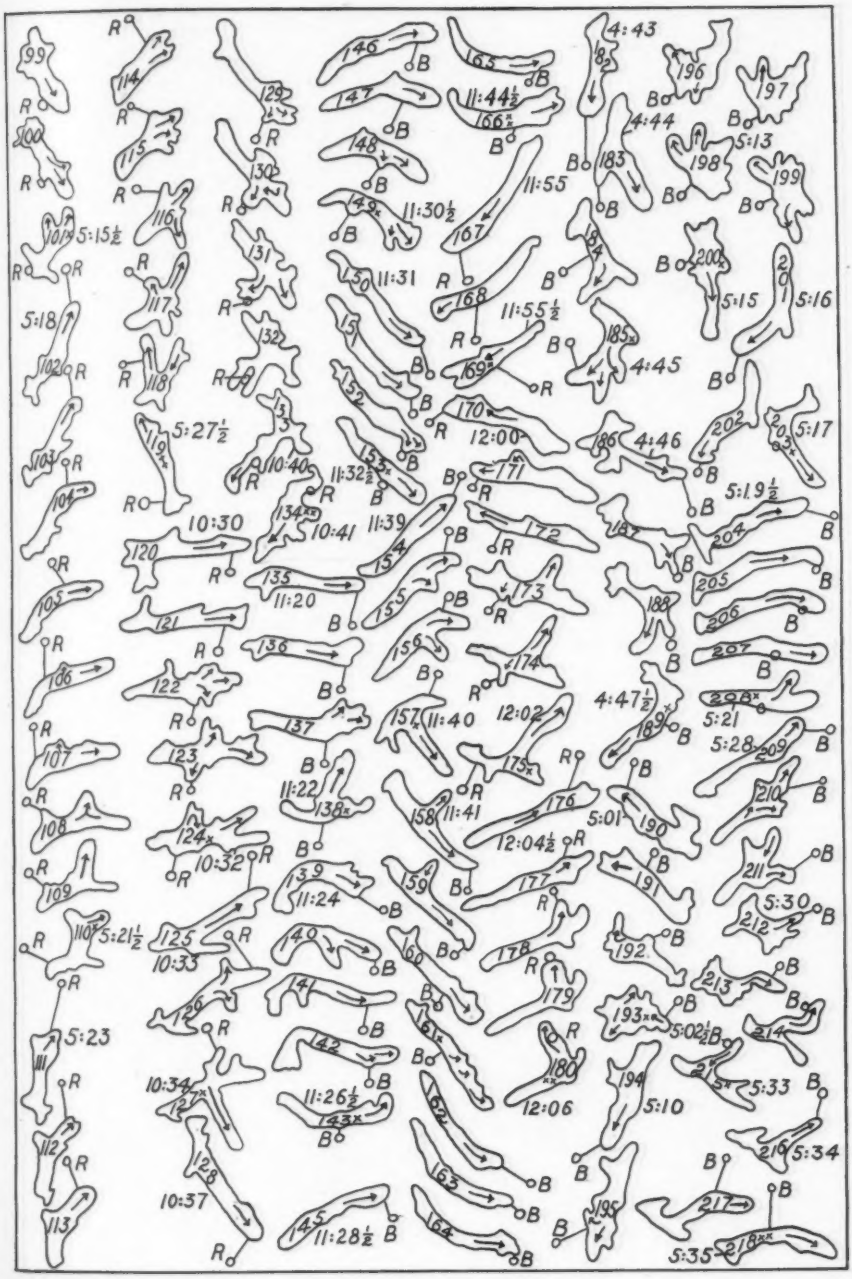

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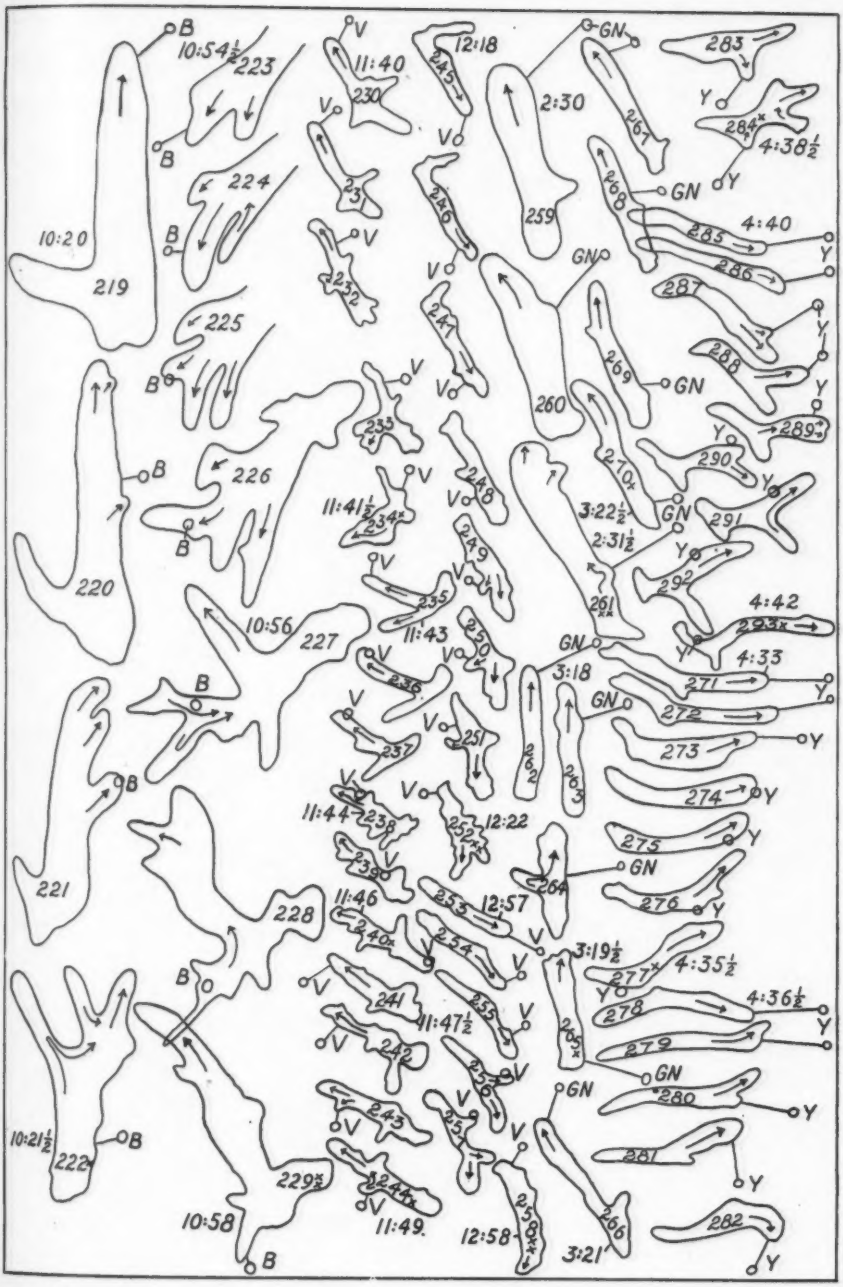

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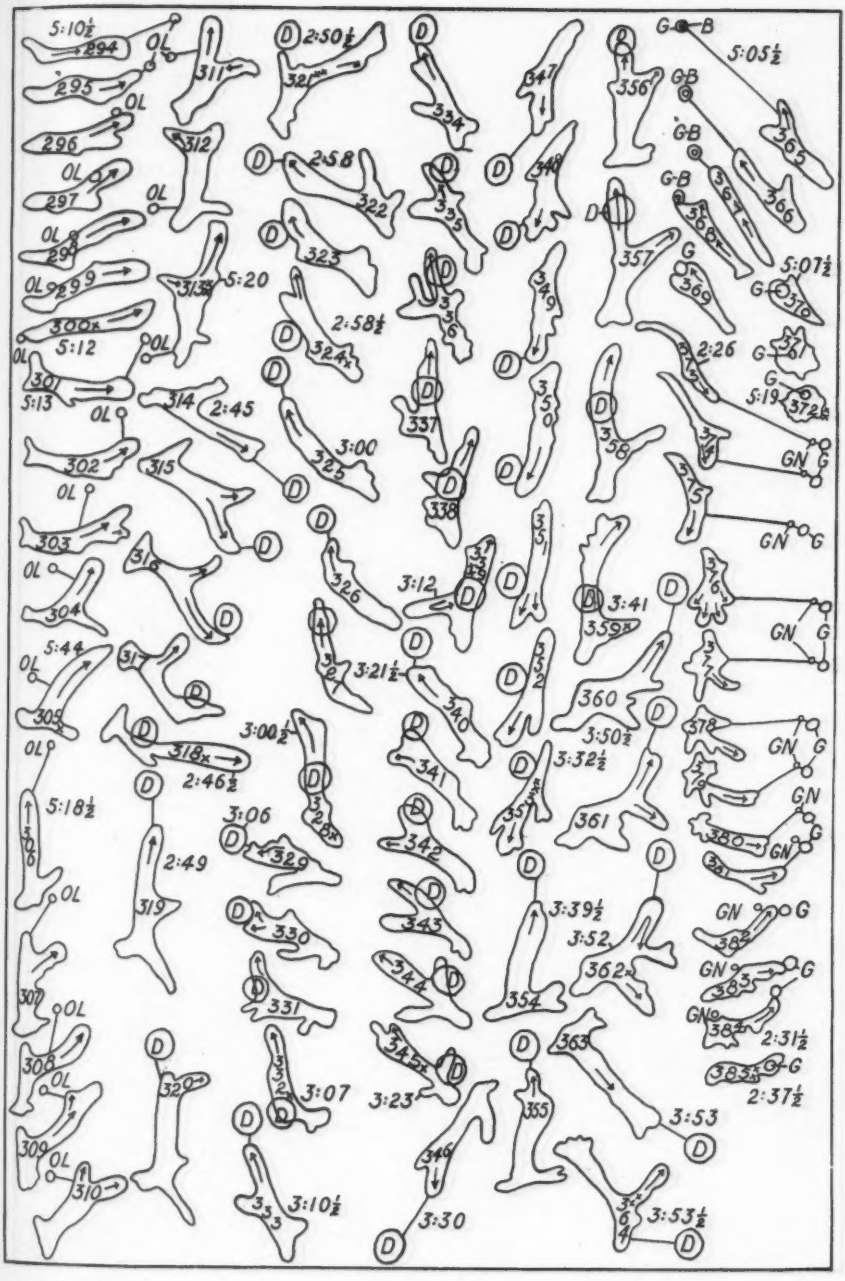

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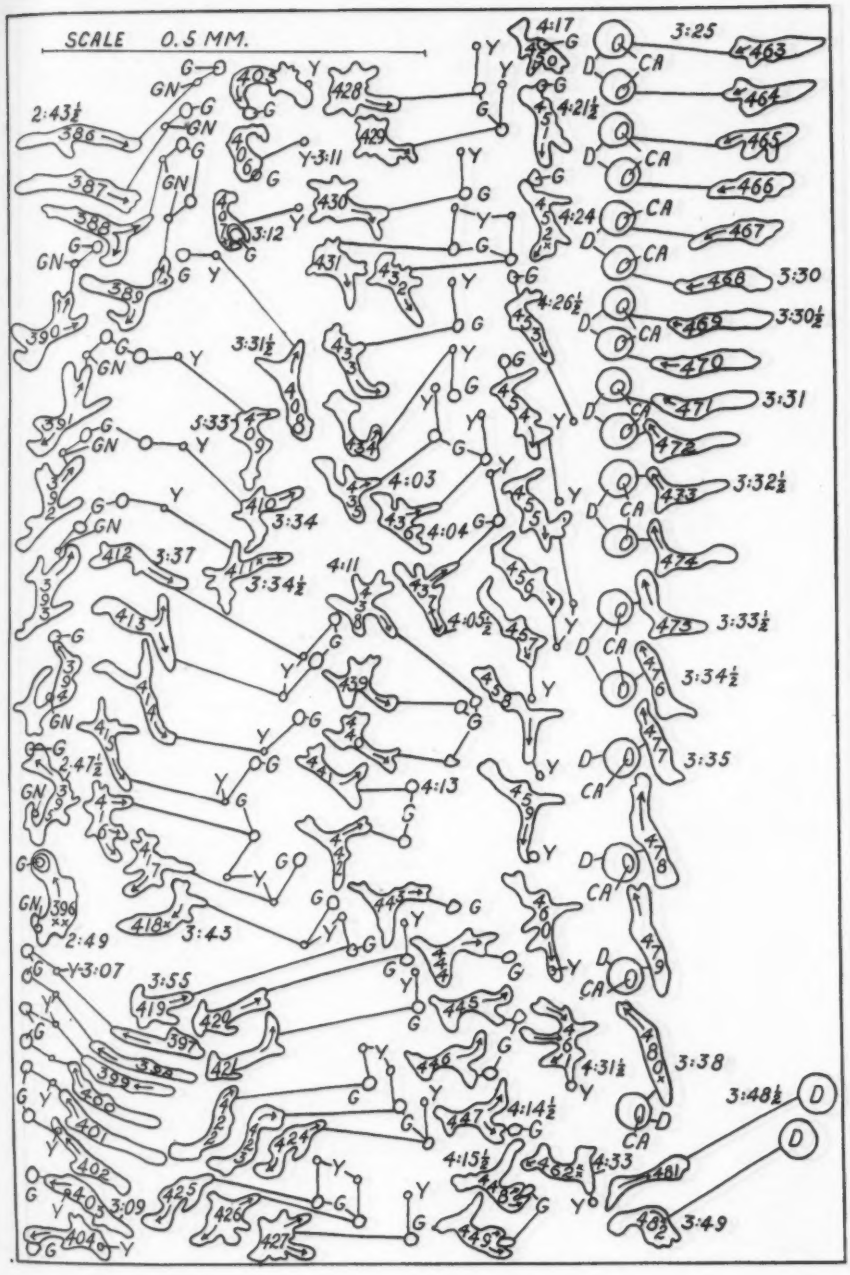
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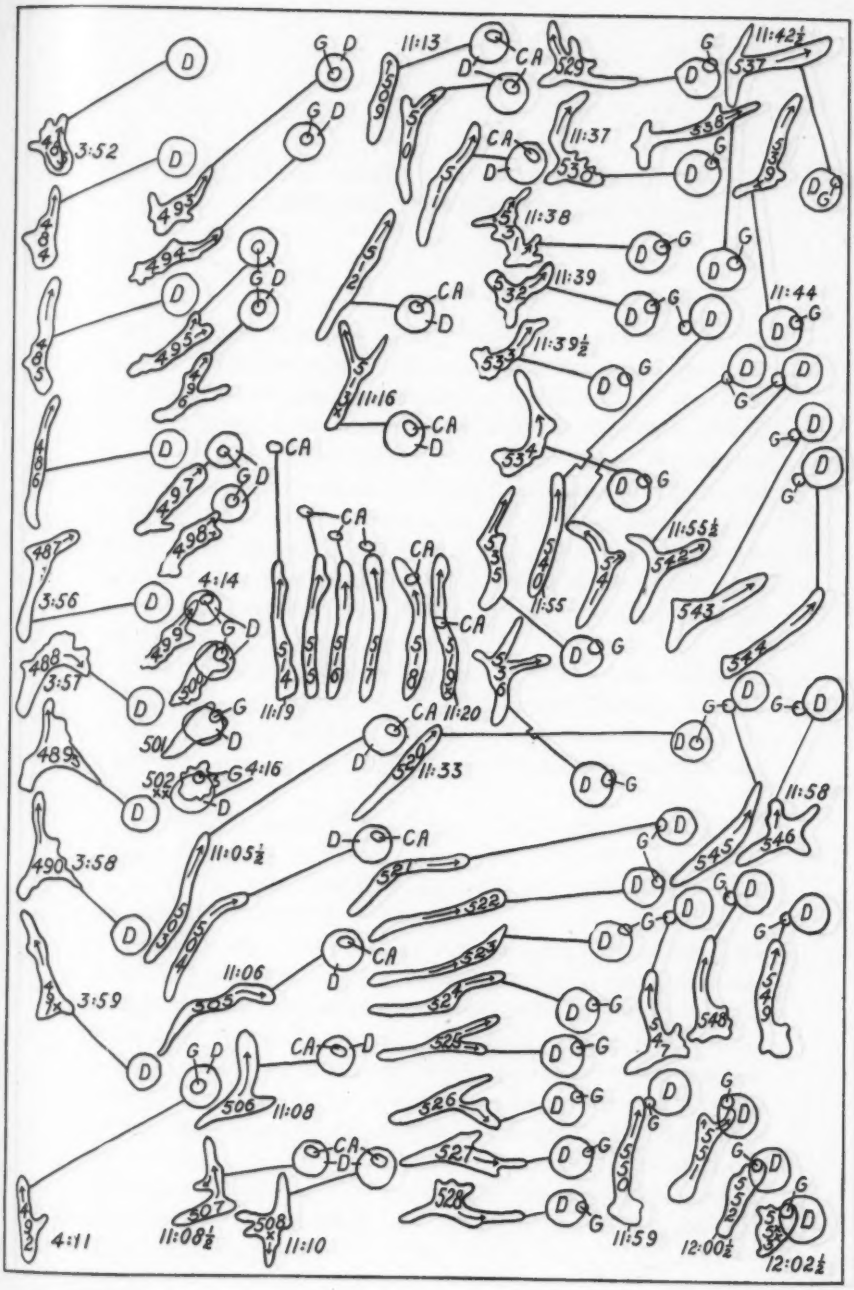
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SUSCEPTIBILITY GRADIENTS IN THE HAIRS OF CERTAIN MARINE ALGÆ.

C. M. CHILD.

WITH FIVE FIGURES.

The colorless hairs or capillary branches so common on the red and the brown algæ are in many cases beautiful objects for the study of the axial susceptibility gradients. A few data obtained during the summer of 1915 at Woods Hole have already been published (Child, '16a). The present paper records the result of further investigation during the summer of 1916 on the physiological polarity of these hairs and includes data on additional species, experimental results on modification of the gradients which answer certain questions raised by the earlier work, and also a correction of the earlier observations concerning one species.

Thus far hairs of the following three types have been examined:

Unbranched unicellular hairs, *Ceramium*, *Chondrus*, *Agardhiella*. The hair is a very slender and delicate outgrowth, sometimes reaching a length of several millimeters, but unicellular.

Unbranched multicellular hairs with basal vegetative tip, *Fucus*, *Castagnea*.

Branched, multicellular hairs with apical vegetative tip, *Chondria*, *Polysiphonia*, *Griffithsia*.

Although this grouping of species according to the form of the hairs does not represent the taxonomic order, it seems the most satisfactory for present purposes since only the hairs are to be considered. In most species examined the hairs are so extremely sensitive that conclusions concerning the existence of a gradient and its direction can be safely drawn only from plants in the best possible physiological condition, collected with the minimum of handling and examined at most within a few hours after collection. Within the first few days in the laboratory, even in running water, the hairs usually die and often drop off, although

other parts of the plant may remain alive and apparently in good condition.

In the earlier observations on the hairs (Child, '16) the extreme sensitiveness and rapidity with which alterations in the gradients may occur was not fully recognized, consequently some of the statements made at that time require correction.

Since the hairs are without pigment and their protoplasm is in most cases almost entirely devoid of visible structure, except under high powers, in the living condition a susceptibility gradient can be made visible only by staining with neutral red or some other vital dye and then killing with the proper concentration of some reagent. As killing agents neutral red, KCN and $HgCl_2$ were chiefly used.

Unbranched Unicellular Hairs.

Ceramium rubrum constituted the chief material for the study of hairs of this kind. In the earlier paper it was stated that "in the long full-grown hairs the susceptibility gradient is distinctly acropetal while in those which are apparently still growing it is basipetal" (Child, '16, p. 104). The later observations indicate that the normal or primary gradient is basipetal in all hairs, but that it may readily undergo reversal. In the observations of 1915 the hairs were first stained with neutral red and then killed in KCN *m*/100. In 1916 it was found that when neutral red was used both as staining and killing agent the more susceptible hairs which died during the first 1 to 2 hours showed almost without exception a basipetal gradient, *i. e.*, death began apically and proceeded basipetally, while among many less susceptible hairs which died later basipetal gradients were much less frequent, many hairs showed no definite death gradient, and acropetal gradients were often observed. Apparently if the neutral red kills the hair rapidly enough the death gradient is basipetal, but where death occurs only after a longer time the original gradient may be obliterated or even reversed. In short the neutral red may obliterate or reverse the original gradient in susceptibility to itself.

Hairs stained for a few moments in neutral red and then killed in KCN usually show a basipetal gradient, but when stained for

an hour or more and killed in KCN the frequency of basipetal gradients is lower and that of acropetal gradients higher, *i. e.*, the neutral red tends to reverse the susceptibility gradient at least to some other agents as well as to itself. Discussion of these and other cases of reversal is postponed to the final section.

Ceramium rubrum is in general highly resistant to depressing conditions and the hairs are much less sensitive than those of many other forms. It has been observed, however, that in plants kept in the laboratory for several days the hairs are usually less susceptible, and acropetal gradients are more frequent than in fresh specimens.

The visible death-changes in these hairs are very similar to those in other cells (Child, '16a, '16b). A slight basipetal staining gradient may or may not be visible. Before the aggregation and decoloration of the protoplasm the usual change in color to deeper red occurs, and this color change commonly shows a basipetal gradient where the death gradient is basipetal. Often death is preceded by plasmolysis which proceeds in the same direction as death and not infrequently a more or less regular gradient in length of the plasmolyzed portions appears, the most apical portion being shortest and the length increasing basipetally (Fig. 1). In other cases the protoplasm may separate into only two or three portions, and death may also occur without preceding plasmolysis. Instead of separating into distinct portions the protoplasm often undergoes a plasmolytic contraction, as a single mass, retracting slightly as a rule from the apical end and basally from one third to two thirds of the hair length. Both the gradient in length of plasmolyzed portions and the difference in apical and basal retraction indicate a graded difference in protoplasmic condition along the axis.

FIG. 1.

The final change in the protoplasm is the apparent collapse of the protoplast or its separate portions and the aggregation into small masses stained almost black. Where the basipetal gradient is well marked this change begins apically and can be followed basipetally along the hair. Where the gradient is obliterated it may begin at almost any level, or at several levels, or may be

simultaneous throughout the length, and where the gradient is acropetal it begins at the basal end of the protoplasm and proceeds acropetally. The deep stain of the masses scattered along the cell rapidly fades and the hair presents almost the appearance of the normal hair, but a careful examination with proper illumination shows the aggregated protoplasmic masses which are still transparent and are visible only because of their refractive index.

The hairs of *Chondrus crispus* and *Agardhiella tenera* are similar in structure to those of *Ceramium* and the few incidental observations made on these species indicate that they behave in essentially the same way. The hairs on freshly collected plants in good condition usually show a basipetal gradient. Most of the hairs die during the first two or three days in the laboratory and acropetal gradients with decreased susceptibility are often observed before they die. Plasmolytic separation or basal retraction of the protoplasm also often precedes death and the final changes are similar to those in *Ceramium*. In the hairs of *Ceramium* and *Agardhiella* the basal retraction of protoplasm often occurs merely as the result of laboratory conditions and before death the entire protoplasm of the hairs may be contracted into a mass one fifth or less of the length of the hair, lying near the apical end and as might be expected, staining much more deeply than in the normal condition. Similar changes probably occur in the hairs of *Chondrus* but have not as yet been looked for.

Unbranched Multicellular Hairs.

Hairs of this kind consist of a single series of cells (Figs. 2 and 3), and in the only two species examined, *Fucus vesiculosus* and *Castagnea tuberculosa*, the vegetative point of the hair is at its attached basal instead of at the free apical end. Because of this fact observations on the susceptibility gradients of these hairs are of particular interest.

In *Fucus* the hairs develop from the inner walls of flask-shaped cavities on the thallus, and later from the walls of the conceptacles. The hairs reach the exterior through the narrow neck of the cavity and form dense tufts, often several millimeters long. As noted above, the growing point of the hair is basal, *i. e.*, next

to the wall of the cavity and is therefore not visible in intact specimens, but the fact that the length of the cells increases from the most basal region visible toward the free end suggests that cell division is basal instead of apical. Fig. 2 shows a hair of *Fucus*, the basal concealed portion being diagrammatically indicated in dotted lines.

In freshly collected plants the hairs are extremely sensitive and even in dilute neutral red death begins within a minute or two, often before the preparation can be placed under the microscope, and in a few minutes the whole hair is dead. In such hairs the gradient is acropetal, *i. e.*, the basal region of the hair is the region of highest susceptibility. The acropetal gradient is very distinct and uniform in the progress of death from cell to cell and in the longer, older cells nearer the free end the acropetal intracellular gradient can usually be seen if death is not too rapid. In the shorter, younger cells an intracellular gradient is usually not visible in the concentrations ordinarily used for killing. The high susceptibility of the hairs makes it impossible to stain with neutral red and then use other agents for killing.

At the time of my first observations on the susceptibility of these hairs I was entirely ignorant of the fact that they grew from a basal instead of an apical vegetative point or "growing tip," but the presence of very distinct acropetal instead of basipetal gradients in all hairs of freshly collected plants constituted practically conclusive evidence for the existence of a basal vegetative region and examination of the cellular structure of the hair and reference to the literature confirmed the conclusion. In this case then the direction of the gradient made possible the immediate recognition of the rather unusual growth form.

FIG. 2.

In freshly collected plants in good condition and very carefully handled, particularly in small young thalli, there may be practically no irregularities or exceptions to the regular acropetal course of death in the hairs of the whole thallus. After a few hours in the laboratory, however, or after exposure to unfavorable conditions, *e. g.*, partial drying at low tide, the change in the condition of these hairs is very striking.

The most susceptible basal region is almost invariably dead, the dead region usually including the two or three most basal cells visible, and the gradient is usually completely reversed in the more distal regions of the hair. Instead of being practically instantaneous, as in the hair when in good condition, staining with neutral red occurs very slowly, and a basipetal staining gradient appears, *i. e.*, the most distal cell stains most rapidly and the rate decreases basipetally from cell to cell. The same basipetal gradient appears in the course of death.

In these hairs with reversed gradient the susceptibility of the most distal region is not much altered; this is the least susceptible region, the last to die in the normal hair and the most susceptible in the hair with reversed gradient, but its susceptibility is very much the same in the two cases. The more basal the level, *i. e.*, the higher the original susceptibility, the greater the decrease in susceptibility afterward, until in the more basal regions the cells which were originally most susceptible of all are dead.

The cells next to these dead basal cells show the structure of dying cells as soon as they begin to stain, *i. e.*, masses of aggregated protoplasm which stain almost black are already present, but include only a part of the protoplasm, and the remainder stains diffusely in the usual way. When the death of such cells is finally completed the remainder of the protoplasm is aggregated into deeply staining masses in the usual way and the diffuse stain disappears. In these cases the death-changes in a single cell may extend over hours. It is a fact of considerable interest that the cellular death-changes resulting from unfavorable conditions in the laboratory or in nature are the same in appearance as those occurring in neutral red and other killing agents.

The very great change in susceptibility in some cells of the hair after reversal of the gradient is indicated by the fact that the cells of the more basal levels which die in one or two minutes in neutral red when the hair is in good condition may survive for twenty-four hours in neutral red after reversal.

What happens in such reversal is apparently this. The hair is very sensitive and under depressing environmental conditions the basal, most susceptible region dies as it does in solutions of killing agents in the laboratory. Next to this region are cells,

originally somewhat less suceptible, and these are "almost" or "partly" killed and their metabolic rate so decreased and protoplasmic condition so altered that they become very insusceptible in killing agents. Still farther distally the effect of the altered conditions is less marked until finally little or no change occurs in the most distal cell. If we now subject a hair altered in this way to the action of a killing agent such as neutral red, KCN, etc., we find that the altered condition appears in the altered susceptibility to the killing agent. The fact that the changes produced by some external agent or complex appear as altered or reversed susceptibility to another is of interest and brings up the question whether the action of the two agents is additive or not. But whatever the nature of the processes, the fact of reversal is sufficiently evident, and, as in other cases, supplements the direct evidence for the existence of a dynamic gradient.

FIG. 3.

Castagnea tuberculosa bears very fragile unbranched multicellular hairs, which like those of *Fucus* develop from a basal vegetative point concealed below the surface of the thallus. Fig. 3 shows the general appearance of the hair, the concealed basal portion being diagrammatically indicated in dotted lines. In my earlier paper (Child, '16*a*, p. 101) I stated that the gradient in these hairs is in general basipetal, but with frequent irregularities, and the earlier observations of 1916 also showed in general a basipetal gradient. In all these cases, however, the plants used had been in the laboratory a day or more and further investigation on fresh material showed that the gradient is primarily acropetal, but very readily undergoes more or less complete reversal as in *Fucus*. In fact, even in freshly collected material apparently in good condition, but with long well-developed hairs, reversal has been observed in many cases.

At the time of the earlier observations I did not realize how readily reversal of the gradient might occur nor was I aware that the vegetative point in these hairs is basal and not apical, and when a well-marked basipetal gradient was found as a rule in plants which had been in the laboratory a day or two, I failed to realize the necessity of checking up the results on perfectly fresh

material. The cells of the *Castagnea* hair attain their full size very near the basal end, so that in that portion of the hair visible in the living plant the cells are all of practically the same diameter and length and it is difficult or impossible to determine the position of the growing point from a gradation in cell size. This case shows how readily incorrect conclusions may be drawn where the material is not in perfect condition. These hairs, like those of *Fucus*, are very sensitive and the gradient undergoes reversal very readily, but there can be no doubt that it is primarily acropetal, not basipetal, as stated in my earlier paper. The hairs are so fragile that they are readily broken or injured mechanically and at least many of the irregularities are due to such injuries.

Branched Multicellular Hairs.

Among hairs of this kind those of *Chondria* have served as the chief material, but the hairs of *Polysiphonia* and *Griffithsia* have also been examined. The hair of *Chondria* develops by repeated apical dichotomy (Fig. 4), and since growth is primarily apical a basipetal death gradient is to be expected. In 1915 hairs of this species were examined by first staining deeply with neutral red and then killing in KCN and the gradient from cell to cell was found to be distinctly basipetal while within single cells an acropetal gradient was almost invariably observed (Child' 16*a*, pp. 105–106). Since these observations were made during the last days of my stay at Woods Hole in 1915 there was no opportunity to repeat the observations with fresh material until the following year. As regards the acropetal cell gradient the results of 1916 are as follows: When the hairs are killed in a high concentration of neutral red alone (*e. g.*, before precipitation in sea-water) both hair gradient and intracellular gradient are very uniformly basipetal. Similarly when the hairs are killed in some other agent (KCN $m/1000$ – $m/100$, $HgCl_2$ $m/50000$) to which a few drops of neutral red have been added to stain the hairs slightly and so make the death-changes clearly visible, both hair-gradient and cell-gradient are basipetal. On the other hand, when the hairs are first deeply stained with neutral red and then killed in KCN or some other agent, the method used in 1915, the

hair gradient is basipetal but the cell gradient is very generally acropetal.

Evidently the acropetal cell-gradient observed in 1915 is not a natural condition but represents a reversal of the cell gradient by neutral red or by other conditions, and this appears as a reversed susceptibility gradient to KCN or $HgCl_2$. These hairs take up neutral red rapidly and stain very deeply, and the axial differences in susceptibility to neutral red within the elongated

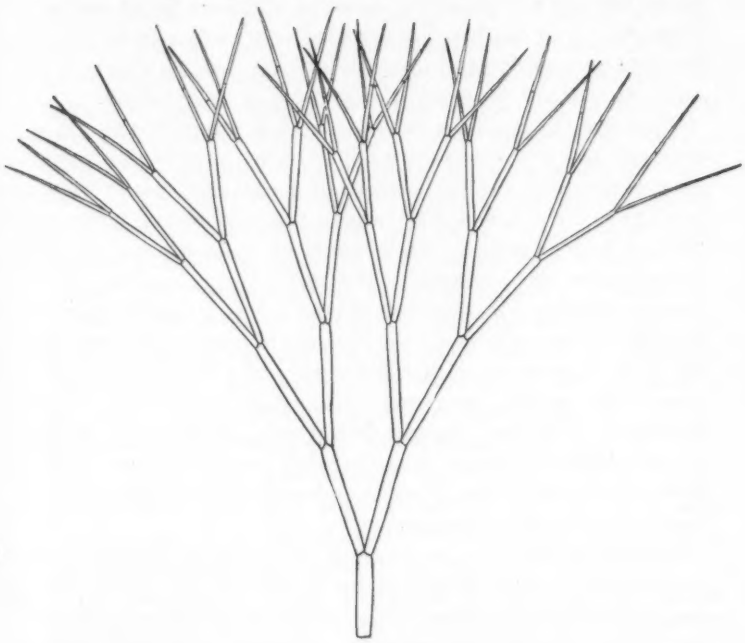

FIG. 4..

cell are sufficient to bring about reversal of the susceptibility gradient after a time. If death occurs early the reversal does not appear, but if the concentration of neutral red is low enough to allow survival for two or three hours in the solution, reversals of the cell gradient begin to appear.

Further observations showed that after two or three days in the laboratory the intracellular gradient was frequently reversed,

particularly in the more basal cells of the older hairs. Similarly in plants found detached reversed intracellular gradients were often observed. Such plants torn loose by the waves and washed into shallow water may be exposed to depressing conditions of temperature, light, or partial drying at low tide. There is of course no way of determining what or how extreme these conditions have been in a particular case but the fact that reversed intracellular gradients appear frequently in the hairs of such plants but are not present in material which so far as can be determined is in the best physiological condition, together with the data on experimental reversal, indicate clearly enough that such reversals are the result of depressing or toxic factors.

Whatever the processes involved in such reversals, they, like other reversals described in this and an earlier paper (Child '16b), are dependent on the preëxistence of a basipetal susceptibility gradient in the cell, and they represent the differential action of external factors within certain limits of concentration or intensity upon this gradient. The difference in susceptibility between different cells, even two adjoining cells, is usually greater than the differences between the two ends of a single cell, and the difference between apical and basal cells of a hair is of course very much greater than any intracellular differences. The persistence of the hair gradient with reversal of the intracellular gradient indicates merely that the relations between the lesser differences in susceptibility are more readily reversed than those between the greater differences.

The establishment of the fact that the acropetal intracellular gradient is a reversal of the original gradient at once raises the question whether the gradient of the hair as a whole can be reversed. Various attempts at such reversal have been made with some success, particularly in the younger hairs, but in the full-grown hairs the axial differences in suceptibility are so great that complete reversal has not as yet been induced and is perhaps impossible.

In *Chondria* the hairs occur in large numbers near the apical ends of branches of the thallus, and in cases where extensive hair-development occurs the hairs appear macroscopically as white tufts covering the branch tips. A well-developed tuft of

this kind is made up of hairs of very different stages of development and physiological age, the most basal hairs being in general the earliest developed and so the largest and the oldest, while the most apical are the latest, smallest and youngest, with intermediate stages between the two extremes. The differences in physiological age in such a group are beautifully shown by the differences in susceptibility, the youngest hairs being most susceptible, the oldest least, with a general gradation in susceptibility between. These age differences appear even in the susceptibility of the apical cells, those cells of the young hair being somewhat more susceptible than those of the old, but this difference between apical cells of young and old hairs is much less than that between the more basal cells. In KCN *m*/1000 for example, apical cells of the youngest hairs die almost at once, those of the oldest hairs after 1–5 minutes, but in the youngest hairs death is complete all the way to the basal cell in 10–15 minutes while in the oldest the most basal cells may live for 2–3 hours. In the young hair the basal cells have not yet had time to become old, while in the old hair the basal cells have reached an advanced stage of physiological senescence, and in the apical cells senescence has progressed to a greater or less extent, but has been counterbalanced to some extent by the periodic rejuvenescence accompanying cell reproduction (Child, '15*a*, Chap. X.). These susceptibility relations between the older and younger hairs of a single group and between apical and basal regions of a single hair constitute a most beautiful and striking example of the course of senescence in plant axes.

The attempts to reverse the whole hair gradient were made with tufts of this sort, consisting often of hundreds of hairs of all ages and stages of development, their attachment to the branch from which they arose being of course undisturbed. In this way it was possible to observe the difference in the reaction of old and young hairs to particular experimental conditions.

The result of a few experiments along this line which serve merely to suggest the possibilities, are briefly stated. Exposure to temperatures of 30°–35° C. for three or four hours killed the youngest hairs completely or all but one or two basal cells, almost without exception. The hairs of medium age and susceptibility,

however, showed a high percentage of complete reversals of the gradient, death beginning basally and proceeding apically, the apical cell being the last to die. In some other hairs of this group partial reversal of the hair gradient occurred, *i. e.*, the apical cell died first, and one or two cells below it in basipetal order, while in more basal regions the progress of death was acropetal, both from cell to cell and within the cells. In the oldest hairs the hair gradient was usually basipetal, in the apical third or half of the hair, *i. e.*, for a length of three to five cells, although the intracellular gradients were often acropetal, particularly in the more basal cells of this region. In the basal half or two thirds of such hairs there was usually more or less reversal of the hair gradient, the basal cell dying first, and one, two or even three cells apical to it dying in acropetal order. The intracellular gradients were very commonly acropetal in this region. In short, by this exposure to high temperature the most susceptible hairs were entirely killed, those of medium susceptibility showed complete or nearly complete reversal and the oldest showed more or less reversal in the basal regions. The susceptibility of all living hairs and cells was of course much lower than that of corresponding hairs or cells in good condition tested at the same temperature, and the susceptibility of the hairs of medium age in which the gradient had been reversed was usually as low as, or even lower than that of the oldest hairs. The experiment shows a gradient in injury by the high temperature both in the single hair and corresponding to age-differences in the tuft, the degree of injury varying in general with the normal susceptibility of different hairs and different levels.

With short periods of exposure complete reversal was obtained in the youngest hairs in many cases, partial basal reversal in those of medium age and in some of the oldest hairs, but usually only intracellular reversal in the more basal cells of the latter. In this case the susceptibility of the youngest hairs was often decreased below that of the oldest.

Staining in neutral red so dilute as to give a barely perceptible yellowish tinge to the water induced complete or nearly complete reversal in some of the youngest hairs, but only more or less reversal of intracellular gradients in the older. It was also

found that in some plants the gradients of the youngest hairs were almost completely reversed after two or three days in the laboratory and after a day or two more these hairs were dead.

Of course intermediate conditions between the normal and reversed gradient are frequently observed. In such conditions the intracellular gradient may be absent or very irregular and the hair gradient may be slight or even absent over a length of several cells. The differences in susceptibility between apical and basal end are, however, so great, particularly in the older hairs, that at the stage when the gradient is obliterated in one region of the hair other portions are either still normal, already reversed or dead.

Where the gradient is obliterated or reversed the hairs show a marked tendency to separate into their individual cells. Apparently the persistence of the orderly multicellular axis is associated with the persistence of the gradient. Other cases in which this separation appears will be described later.

The hairs of *Polysiphonia* are similar in structure and growth to those of *Chondria*, but in *P. variegata* and *P. fibrillosa*, the only species examined thus far, they are considerably more susceptible than the *Chondria* hairs and therefore not so favorable for experimental work. The gradient is primarily basipetal both in the hair in general and in the cell, and while more or less reversal has sometimes been observed, experimental reversal has not been attempted.

The hairs of *Griffithsia* differ from those of *Chondria* chiefly in the whorled arrangement of branches (Fig. 5) and as we should expect, since growth is primarily apical, both general and intracellular gradients are basipetal when the hair is in good condition. After two or three days in the laboratory, however, irregularities and reversals appear, and the hairs soon separate into cells or fall off entire.

Discussion.

It is evident that at least the hairs of these three kinds among the algæ show axial susceptibility gradients like those in the vegetative axes of the thalli. Moreover, these gradients show a direct relation to the location of the vegetative point, the region

of highest susceptibility being in general the region of the vegetative point, at least when the hair is in good condition.

In *Fucus* and *Castagnea*, for example, where the vegetative point is basal, the gradient is normally acropetal, while in all other forms examined the vegetative point is apical and the gradient normally basipetal.

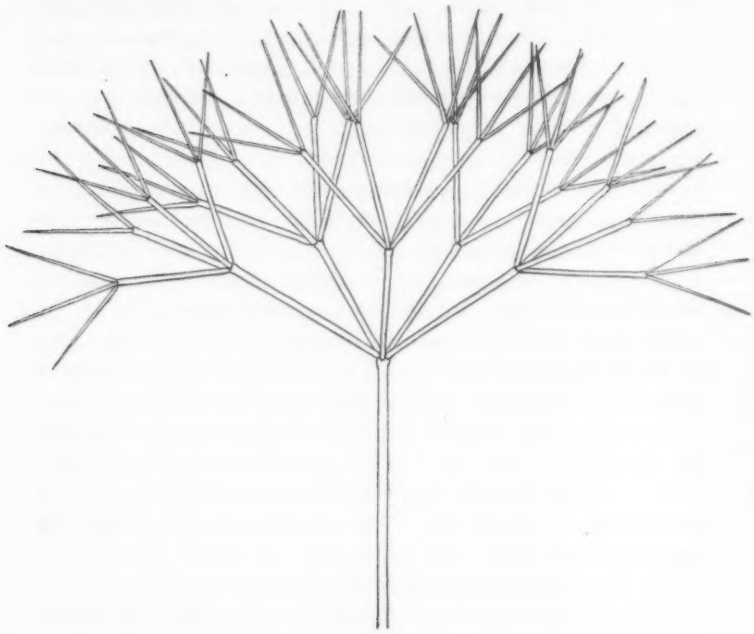

FIG. 5.

Under depressing conditions which are not extreme enough to kill rapidly the gradient may be more or less completely obliterated or even reversed, and such changes are of course a consequence of the differential susceptibility originally present. Obliteration or reversal of a gradient by external factors means simply that a region of highest susceptibility or metabolic rate, is affected by the external factor to so much greater extent than a region of lower susceptibility or rate that the original differences are obliterated or even reversed. In some cases an external

agent may reverse the susceptibility to itself, as in the case of neutral red, and it is also possible in many cases to determine the effect on susceptibility of one agent by using another for killing after exposing to the first. The whole complex problem of the nature of the action of external agents of different kinds on protoplasm is involved in these data on susceptibility, but even though we are far from the solution of that problem we can establish certain general laws of susceptibility as characteristic features of a physiological axis, *i. e.*, as an expression of polarity.

When the gradient is obliterated or reversed multicellular hairs often separate more or less completely into individual cells. The persistence of the hair as a multicellular order seems to be associated with the existence of the gradient. It will be shown elsewhere that this is true, not only for these hairs, but for the vegetative axes of certain species of algæ. Tobler ('02, '04) has observed in various species of the red algæ this tendency to separate into cells under unfavorable conditions and has used it as a method for inducing experimental reproduction. In the isolated cells of the hairs reproduction can scarcely be expected, but the relation between morphological order and physiological correlation is evident. After obliteration or reversal of the dynamic axial gradient the morphological order soon ceases to exist except where the structural stability is high.

A question of some interest concerning what may be called the death-level arises particularly in connection with the *Chondria* hairs. The young highly susceptible hairs of *Chondria* die without perceptible reversal of the gradient under conditions which bring about complete reversal in the hairs of medium age and some reversal of intracellular gradients with perhaps partial reversal of the hair-gradient in the oldest hairs. To reverse the gradient before death in the youngest hairs less extreme conditions must be used. These and various other facts suggest the possibility that the death-point, or more properly speaking, the death-level is not necessarily the same quantitatively for more active and less active protoplasm of the same kind and for more and less rapid killing, in other words the death-level may be relative rather than absolute. With our present lack of knowledge this remains merely a possibility.

This possibility has already been suggested in connection with certain observations on susceptibility in the lower animals (Child, '13, pp. 118–119) and it seems worth while to call attention to it in this connection. Actually the death-level, whether relative or absolute, must be a resultant of various component factors such as aggregate condition of colloids, permeability, rate of metabolism or of certain reactions, etc., and there can be little doubt that different agents and conditions act primarily or chiefly on different factors in this complex. But since these are factors in determining the behavior of a system, *i. e.*, are correlated, not independent, changes in one cannot proceed beyond a certain limit without inducing changes in the others. This being the case, the death-level as a quantitative level at which persistence of the system as a whole becomes impossible, must be for each particular case of death a more or less definite level or region of the curve which represents the quantitative changes in the activity of living protoplasm or its component factors. On the other hand, the previous condition of the system and the character and rate of action of the killing agent may play a rôle in determining this level in different cases. If this suggestion is correct, then regions or cells of different susceptibility in the hairs do not necessarily attain exactly the same condition at the point of death, and the condition in a particular cell or region may differ according as it is killed rapidly or slowly.

Since the hairs represent real physiological axes, similar to other plant axes the question of their physiological relation to other axes must be raised. Unquestionably the development of a hair axis is a form of reproduction in a protoplasm specialized in some way. As a reproductive process it must result from physiological isolation (Child, '11, '15*a*, Chap. IX., '15*b*, Chap. V.) and, as I have pointed out, physiological isolation and reproduction may occur, not only as the result of increase in size but also under depressing conditions in consequence of decreased dominance, *i. e.*, decrease or obliteration of a preëxisting axial gradient. Hairs frequently appear on young apparently vigorous plants, but they are often very characteristic of advanced vegetative stages or of plants which seem to be in poor condition. In *Agardhiella*, for example, I have frequently observed that parts

of the fronds bearing hairs show a lower susceptibility than other parts (Child, '16b). The hair is an axis arising from a cell of a previously existing axis. Like any other physiological axis it represents a metabolic gradient and originates as such a gradient in a cell which, though apparently specialized in certain respects is still capable of undergoing growth and division. In species where the hairs are not extremely sensitive to laboratory conditions it will probably be possible to control their occurrence and development by controlling the metabolic condition of the plant and in this way to learn something of their physiological relation as axes to the other axes of the plant.

Summary.

1. The unbranched unicellular hairs of *Ceramium*, *Chondrus* and *Agardhiella*, the unbranched multicellular hairs of *Fucus* and *Castagnea* and the branched multicellular hairs of *Chondria*, *Polysiphonia* and *Griffithsia* all show an axial gradient in susceptibility to various agents.

2. In the hairs of *Fucus* and *Castagnea* in which the vegetative point is basal the normal hair gradient is acropetal while in the other species where growth is primarily apical the gradient is basipetal, *i. e.*, in all cases the region of highest susceptibility is the region of the "growing tip."

3. Obliteration or the reversal of the susceptibility gradients has been observed or induced in the hairs of *Ceramium*, *Fucus*, *Castagnea* and *Chondria* and to some extent in those of *Griffithsia*. These changes in the gradient are dependent on the differential susceptibility to various depressing agents and conditions, which is a feature of the normal gradient. In general a high concentration or intensity kills so rapidly that reversal of the gradient cannot be demonstrated, but with lower concentrations or intensities the original differences in susceptibility may be obliterated or reversed before death occurs. In some cases a particular agent may reverse the susceptibility gradient to itself, and in others a reversal of the gradient produced by one agent may be demonstrated by the differential susceptibility to another.

4. The hair, like the vegetative portions of the plants, represents a physiological axis and the susceptibility gradient is one

aspect of the difference in metabolic and protoplasmic condition along the axis, in other words of the physiological polarity. The multicellular hairs often tend to separate into their cells when the gradient is obliterated or reversed, and the loss of the hairs under laboratory conditions or in other unfavorable environment is associated with the change in the gradient which usually occurs under such conditions.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
November, 1916.

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THE SALT CONTENT OF NATURAL WATERS IN RELATION TO RHEOTAXIS IN ASELLUS.[1]

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Much of my work on rheotaxis of the isopod *Asellus communis* Say has been based on the fact that pond and stream mores of the same species give different rheotactic reactions. Among other differences the pond mores have a low, and the stream mores, a high degree of positiveness ('12). I have wished for some time to determine whether there were differences in the salt content of the two classes of habitats that might account for the differences in behavior. Upon my return to the Chicago area after an absence of three years, I hastened to make this survey. The subject is the more interesting since experiments in the meantime have shown that the rheotactic reaction may be controlled by various salts when present in sufficient concentration.

Through the kindness of Mariner and Hoskins, to whom I was already indebted for similar analyses, the spring and autumn salt content was determined for County Line Creek, near Braeside, Ill., and for a point near Osborn, Indiana (Slough 93; Shelford, '13). Many of the isopods used in experimental work have been obtained from these habitats. The analyses are from approximately maximum-minimum water level for the year 1916 and are given in detail in Table I.

Of the elements found, calcium and magnesium are known to decrease the positiveness of rheotaxis in *Asellus* when present in sufficient concentration. If the salt content is at all responsible for the low positive rheotactic response of pond isopods, its effect should be most marked in the most concentrated water analyzed. This water contained 228.4 mg. of calcium per liter of water, the

[1] I am indebted to the Elizabeth Thompson Fund and to the Bache Fund of the National Academy for money grants and to Mariner and Hoskins for water analyses without charge; without their aid this work could not have been done.

TABLE I.

SHOWING MAXIMUM-MINIMUM WATER LEVEL ANALYSES OF WATER FROM COUNTY LINE CREEK, BRAESIDE, ILL., AND SLOUGH 93, OSBORN, IND.

The solids are shown in parts per million by Mariner and Hoskin's analyses and the gases in c.c. per liter.

	Stream.		Pond.	
	Maximum.	Minimum.	Maximum.	Minimum.
Nitrogen as free ammonia	0.024	0.023	0.320	0.160
Nitrogen as albuminoid ammonia	0.150	0.098	0.160	0.288
Nitrogen as nitrates	None	0.700	0.840	0.800
Nitrogen as nitrites	0.0015	0.002	0.0012	None
Chlorine	5.000	12.000	72.000	277.000
Iron	0.200	1.400	0.500	1.400
Calcium	50.300	61.850	72.500	228.400
Magnesium	0.360	33.700	0.120	45.300
Sodium	45.2	8.0	45.200	116.700
Potassium	2.700	Trace	6.900	Trace
Free oxygen	6.8	3.0	5.1	1.6
Free carbon dioxide	3.2	2.5	8.6	11.7
Half bound carbon dioxide	79.5	82.6	113.4	119.6

equivalent of the amount of calcium contained in 0.011 normal solution of calcium chloride. Supposing for the moment this calcium were the only cation present in the water it would have no depressing action on rheotaxis.

Witness:

Isopod 146 gave a rheotactic reaction of 30 per cent. +, 50 per cent. −, 24 per cent. α when first tested and after being in 0.05 normal solution of calcium chloride for five days the rheotactic response was 90 per cent.+, 10 per cent. −.

Isopod 147 when first tested gave 60 per cent. +, 10 per cent. −, 30 per cent. α and after 48 hours in a similar solution of calcium chloride gave 40 per cent. +, 30 per cent. α, 30 per cent. 0.

Three other isopods (Nos. 175, 176, 177) after 10 days in the same strength calcium chloride solution gave an average response of 37 per cent. +, 30 per cent. α, 33 per cent. 0 while the average rheotactic reaction of 253 untreated isopods from similar environmental conditions was 31 per cent. +, 45 per cent. −, 22 per cent. α, 2 per cent. 0.

But calcium is not the only cation present. Magnesium, a less powerful isopod depressant than calcium, is found to the extent of 0.0037 normal solution. If at this strength magnesium

retains its depressing power, which laboratory tests similar to those just cited renders improbable, its action would be offset by the sodium which has an antagonistic effect and which is present in sufficient quantity to neutralize any possible depressing action of the nagnesium and to antagonize partially the action of the calcium, if that were needed.

Theoretically then, from the analyses of salt content, one would not expect the pond water to be less favorable for positive rheotactic reaction than the water from the stream; and this expectation was verified by laboratory tests. Stream isopods were kept in the laboratory for three days in water brought from their stream habitat. Then they were divided and part were put into pond water from the Osborn, Indiana, pond. Both lots of isopods were kept under identical conditions of temperature, aeration, and food supply for twenty days, during which time the eighteen tests shown in Fig. 1 were made. The graphs there show the percentage of positive reactions given daily by groups of five isopods chosen at random from the stocks under observation. The variation in positiveness is due in part to this chance selection of individuals for the daily tests ('13*a*) and in part to the setting in of an abnormal breeding season, brought on by the transfer from freezing stream water to a laboratory of about 15 degrees centigrade. The decline of positiveness is the usual effect of the breeding season.

For the eighteen days when comparative tests were made the stream isopods in pond water averaged 73 per cent. positive while their mates in stream water were 70 per cent. positive. This means that the difference in salt content of the two waters did not affect rheotaxis.

The most obvious difference in the two environments is the difference in the oxygen and carbon dioxide tension in the water. In the more extended work of five years ago, the oxygen tension of County Line Creek was found to lie normally between 5 and 10 c.c. per liter and the carbon dioxide tension was about 2 c.c. per liter. In the ponds the oxygen was usually under 3 c.c. per liter and the free carbon dioxide usually over 10 c.c. per liter. The amount of half bound carbon dioxide is also consistently greater in the pond than in the stream.

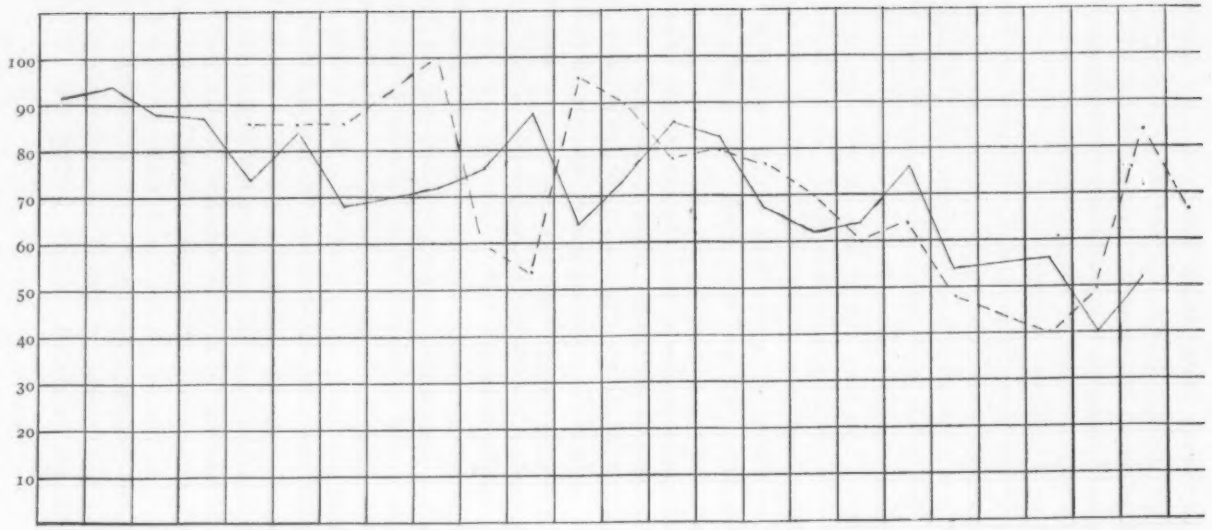

FIG. 1. Showing the result of rheotactic tests of stream isopods kept in the laboratory in stream water (solid line) and in pond water (broken line). As far as known the kind of water was the only factor varied. The abscissæ give the positive rheotactic reaction in percentage of the total trials for the day. Each space in the ordinates represents one day.

The rheotactic reaction of *Asellus* can be experimentally controlled by varying the oxygen and carbon dioxide tension within the limits found in the two classes of habitats. This is not true of similar variation in salt content, or in any other factor or group of factors yet studied.

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AN EXPERIMENTAL STUDY OF THE REACTIONS OF THE HORNED LIZARD, PHRYNOSOMA MODESTUM GIR., A REPTILE OF THE SEMI-DESERT.[1]

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I. INTRODUCTION.

The species of *Phrynosoma* which is the subject of these observations is one of the smaller and less conspicuous members of the genus, and is one of the commoner "horned toads" in New Mexico, which lies at the center of its rather restricted range (Ditmars, '08). This species, *Phrynosoma modestum* Gir. (Girard '53) has been confused to a certain extent in the literature (Herrick, *et al.*, '99) with another small member of the genus, *Phrynosoma platyrhinos* Gir. (Girard, '53) which extends into New Mexico from the northwest, but a reference to the original descriptions differentiates very clearly between the two groups.

1. *General Habits.*

Near Albuquerque, where the individuals observed were taken, the species is widely distributed over the sparsely vegetated "mesa" (Watson, '12) or clinoplane region varying in altitude from 1,500 to 2,200 meters and having a rainfall of approximately 30 cm. annually. Bailey ('13) gives the distribution of this animal as the Lower and Upper Sonoran Zones in New Mexico. In the valley of the Rio Grande, just below the "mesa" region, and in

[1] Contribution from the Zoölogical Laboratory of the University of Illinois, No. 86.

the Sandia Mountains bordering on the upper side the very different *Phrynosoma douglasii ornatissimum* prevails. This species is also found on the mesa but in much smaller numbers, and is said by Bailey (*l.c.*) to occur in the Upper Sonoran Zone. The whole region under consideration is given in his map as the Upper Sonoran Zone, with the exception of the Sandia Mountains, which include the Transition and Canadian Zones, in both of which the above variety of *P. douglasii* is found. Both mountains and valley are considerably damper than the mesa, the former on account of the greater annual rainfall, and the latter on account of the proximity of the Rio Grande and the high water table (10 cm. to 5 m. from the surface of the soil (Nelson, *et al.*, '14)). The distribution of these species seems to depend, not primarily on air temperature or on the factors which define the Life Zones of Merriam, although the upper limit of *P. modestum* approximately coincides with the upper border of the Upper Sonoran Zone, but upon factors which are in agreement in the river valley and the mountain range. Ants, the principal natural food of *P. modestum*, are more abundant in the mesa region, but this may be merely a secondary factor as far as distribution is concerned, as the animals eat and thrive well upon small beetles, plant bugs, grasshoppers, etc. The distribution of the species coincides with the grassy step formation of Watson ('12) falling in Shelford's ('13*a*) third division, *formations of savannas and grasslands*.

In captivity, it was observed that the lizards ate readily any living and moving insect or other animal of sufficiently small size. The animals have been observed even to ingest lead shot kept in motion by being rolled down an inclined plane, and occasionally snap at moving sand grains. The attempts of a medium-sized *P. douglasii* to swallow an earthworm of three times its own length were rather interesting. The lizard braced itself by means of its legs and attempted to pull the worm apart, but finally succeeded in swallowing it whole, shaking it in the process much after the manner of a dog shaking a snake. Large woolly caterpillars, grasshoppers of considerable size and other seemingly difficult objects were swallowed with comparative ease. The animals were never observed in the act of drinking and evidently obtained the amount of moisture required with the food.

Phrynosoma modestum is not, essentially, a desert and heat-loving species, although it seems more tolerant of desert conditions than *P. douglasii*. It appears in the greatest abundance in the early summer months, and during the early autumnal rainy season when the aërial temperature does not exceed 32° C., and when the temperature of the substratum in the sunlight is not over 38°. During these periods the animals actively move about all day, remaining during the night in protected nooks under bushes or in the burrows of other small animals, or occasionally buried in the loose soil. When the daily maximum temperature becomes greater the period of activity begins earlier in the morning and is terminated toward noon, when the temperature rises. During the heated part of the day the lizard is at rest, nearly, if not quite, covered by the loose soil or sand. The method by which this position is reached is very characteristic. The snout is directed downward and moved from side to side, the body extremely flattened, while the legs take part in a rapid horizontally clawing movement. The net result of this series of motions is to cover the animal with the loose soil, the depth varying according to the character of the soil, the individual, etc. When the temperature is high, sunlight is avoided and burrowing is more likely to take place in the shade of vegetation, a bank of soil or a rock. Other lizards have been observed to burrow in like manner as a means of escape from enemies.

2. *Color and Color Changes.*

The color of the animal generally resembles rather closely the color of the substratum, but this is not always the case. In general, individuals observed after a rain are darker in color (the soil is also darker) and very much lighter on a hot dry day. Experiments have shown, in the case of *P. douglasii*, that a rise in temperature, darkness or an increase in the evaporating power of the air causes a centripetal migration of the melanophoric pigment, while the opposite conditions induce a corresponding movement. Within the limits of the changes occurring in the habitat, the variation in the evaporating power of the air is the most potent of the above factors of color change. No direct connection between the color of the animal and that of the substratum has been confirmed experimentally.

3. *Behavior in Captivity.*

In captivity they are rather inactive, except when living food animals are introduced into the cage, when they may become very active, or when otherwise disturbed. They spend a great deal of time with the belly flattened to the ground, with the head lowered and the eyes closed. When the sun is not shining directly on the cage the majority of the animals are oriented toward the source of light (*e. g.*, a window) and often climb up on the side of the cage toward the light. On a cool morning they often orient themselves with respect to the sun so that the surface of the back is as nearly as possible perpendicular to the path of the rays. This is accomplished by tilting the body sidewise. When the air temperature is in the neighborhood of 30° or above direct sunlight is avoided,and the animals are active only when the cage is shaded. This is not in line with reported field observations on *Phrynosoma*, most of which seem to be based on the true desert species.

The horned lizards used for the following experiments were taken near the lower edge of the "mesa" (altitude 1,700 m.) just east of the city of Albuquerque, New Mexico, and near the campus of the University of New Mexico, about June 10, and shipped by express to the University of Illinois. They were kept, during the experiments, in two lots, one (cage No. 1) in a cage on the floor of a northeast second-floor room of the vivarium building, where the sunlight reached the cage but a few hours each forenoon. The evaporation in this cage,.as measured with the Livingston porous cup atmometer averaged .5 c.c. per hour or 12 c.c. in 24 hours. The other lot was kept in a similar cage in the ground-floor greenhouse, where they were subjected to the direct rays of the sun until 2 P.M. The evaporation was here .8 c.c. per hour. The animals were fed black ants the greater part of the time, with occasional feedings of miscellaneous insects from net sweepings of vegetation near the building.

II. Experimental Results.

1. *Air Humidity Gradients.* (*Evaporation Varied by Differences in Air Humidity.*)

A large number of experiments (120) were performed with the object of determining the optimum air evaporation and the

reactions of the animals to air of different evaporating powers. The evaporation gradients were established by varying the humidity, the temperature and the rate of flow of the air to which the animals were subjected. The apparatus used in the gradient experiments was essentially the apparatus described by Shelford and Deere (1913) and Shelford ('14*a*) with the following modifications. The source of the air was the compressed air system of the University of Illinois. The measurement of the flow of the air was made more accurate by the insertion in the supply line of delicate draft gages described by Hamilton ('17). The gradient cages used in this series of experiments were also larger ($45 \times 8 \times 5$ cm.) to accommodate the larger animals, and the floors were covered with a layer of sand 1 cm. in thickness.

The experiments ordinarily covered each a period of twenty minutes, each space on the recording blank representing ten seconds, as indicated in the accompanying Chart I. The figures at the top of each column represent, respectively, the relative humidity, computed from readings of standard wet and dry bulb thermometers, and the evaporation per twenty-minute period, measured with porous cup atmometers, of the air supplied the corresponding third of the gradient cage. In the graphically recorded experiments an animal was placed in the cage, generally in the center section, and its movements recorded by means of tracings. In the statistically recorded experiments ten individuals were distributed through the cage and records of their positions made at the indicated intervals. Experiments designated by the same number were performed consecutively and with the *same* animal or group of animals. Otherwise the numbering is entirely arbitrary. For a more complete account of the method of recording, etc., see the explanation of Chart I.

In Experiments 12, 13 (Table I.) ten individuals were placed in the gradient cage and readings as to position were taken every thirty seconds, the final results being expressed in percentage figures. Experiment 12 was performed with individuals from Cage No. 1, in which the temperature varied between 24° and 35°, while those used in experiment 13 were from Cage No. 2, where the temperature rose to 40° during the warmer parts of the day. There was no marked difference in the behavior of the two

TABLE I.

SHOWING THE REACTIONS OF *Phrynosoma* IN GRADIENTS OF AIR HUMIDITY.

The method of procedure here was the same as that pursued in the experiments illustrated in Chart I., except that ten individuals were used in each experiment and numerical readings were taken at half-minute intervals. The summation of the results is indicated in numbers and in percentages at the foot of the columns. See explanation accompanying Chart I., and page 102 for discussion.

Minutes.	Experiment 12. Humidity.			Experiment 13. Humidity.			Experiment 15. Humidity.		
	98%.	57%.	6%.	6%.	57%.	98%.	93%.	52%.	5%.
	0.3.	0.8.	2.0.	2.0.	0.8.	0.3.	0.2.	0.5.	1.8.
	3	3	4	3	3	4	3	4	3
1	4	2	4	2	5	3	1	6	3
	3	4	3	1	7	2	2	4	4
2	4	3	3	1	7	2	2	5	3
	4	4	2	2	7	1	3	5	2
3	5	4	1	4	5	1	3	5	2
	5	4	1	3	3	4	3	4	3
4	5	3	2	1	3	6	3	5	2
	5	4	1	2	4	4	2	5	3
5	4	6	0	2	2	6	2	6	2
	4	6	0	4	2	4	2	6	2
6	3	6	1	3	3	4	4	5	1
	5	4	1	1	4	5	4	5	1
7	5	4	1	2	3	5	3	5	2
	5	5	0	2	3	5	4	4	2
8	5	4	1	1	3	6	4	4	2
	5	4	1	3	2	5	1	5	4
9	4	5	1	3	5	2	3	5	2
	2	5	3	2	5	3	1	6	3
10	6	2	2	2	3	5	1	5	4
	4	3	3	4	4	2	0	5	5
11	4	4	2	4	4	2	1	5	4
	5	4	1	2	4	4	1	5	4
12	5	4	1	3	4	3	1	4	5
	3	5	2	5	2	3	0	5	5
13	3	5	2	3	3	4	3	3	4
	3	4	3	1	4	5	1	7	2
14	4	4	2	4	4	2	1	8	1
	3	5	2	4	3	3	2	8	0
15	4	4	2	3	2	5	1	8	1
	5	4	1	2	5	3	2	8	0
16	5	3	2	1	4	5	1	8	1
	6	3	1	3	2	5	2	6	2
17	5	2	3	2	4	4	1	8	1
	4	3	3	2	5	3	1	8	1
18	3	6	1	4	5	1	1	7	2
	5	4	1	1	7	2	0	9	1
19	5	2	3	0	7	3	3	7	0
	5	4	1	2	5	3	2	7	1
20	5	3	2	2	5	3	3	7	0
	172	157	71	97	160	143	89	218	93
	43%	39%	18%	24%	40%	36%	22%	54%	24%

groups in this or in other experiments. In both cases the driest air was avoided, and the greatest number were found in the central portion of the cage, where the evaporation was .84 during a twenty-minute period. Many other similar experiments gave similar results.

Experiment 15.—This experiment did not show as much difference between the extremes but showed a great percentage in favor of the central section of the gradient cage in which the evaporation was much lower than in the preceding experiment (.5).

Summary of Air Humidity Gradient Experiments.

The animals ordinarily avoid air of excessive evaporating power, or, if they remain in such air, are stimulated in such a way that attempts to escape are made, or are stimulated to burrowing or digging activities. The net result of either reaction, if successful, is an escape from the unfavorable environment. As nearly as could be determined from the data at hand the optimum evaporation for the average animal lies between .5 c.c. and 1.0 c.c. expressed as the evaporimeter reading for a twenty-minute experimental period. There is a considerable variation in individual behavior and that of any individual will vary from time to time, due, presumably to changes in physiological condition. The normal evaporating power of the air in the natural habitat during the months of April and May varies from .15 c.c. twenty-four hour average on a rainy and cloudy day, to 1.0 c.c., on a bright clear day when the maximum temperature is in the neighborhood of 30°. Although day and night observations have not been made separately it is evident that the average evaporation for the daylight hours would be somewhat higher than the figures just given.

Animals kept for a long time in abnormally dry atmospheric conditions evidence a marked degree of uneasiness, indulge in many spasmodic movements, and finally either burrow or apply the body closely to the substratum. They are, however, able to endure long exposure to dry air without death. A 2.5 gram animal was exposed to a constant current of air with an evaporation of 2.4 c.c per twenty-minute period for a week before death took place. Death in this case was also probably largely due to

starvation, as it was difficult to induce the animal to take food under the conditions of the experiment. The weight in this time was reduced to 2 grams.

The broad limits of the evaporation optimum make it rather unlikely that this factor is the principal one in determining the local distribution of the species. It would not be expected that this would be the case in a reptile from such a habitat, where the evaporating power of the air is subject to such great variations in the course of twenty-four hours. Reptiles, also, are, in general, especially adapted to withstand a considerable amount of drouth, the water loss through the integument being very slight and that through the feces and urine almost negligible, as compared to the similar losses of other animals.

2. *Air Temperature Gradients.* (*Evaporation Varied by Differences in Air Temperature.*)

Experiments with an air temperature gradient were carried out in the same apparatus as the previous experiments, the air passing through the cage being heated or cooled by passing through coils immersed in water of high or low temperature. The air came directly from the storage tank and was unmodified except as above noted and flowed at the same rate as in the humidity experiments. The experiments (34, *a*, *b*, *c*) summarized in Table II. together with the many others performed in the same manner show the optimum to be between 33° and 38° when the substratum is not cooled, *i. e.*, when the temperature of the substratum varies approximately with the air. When the substratum is cooled the optimum more closely approaches 30°, which corresponds, under the conditions of the experiment, to air with an evaporation of 1.0 c.c. for the experimental period. Both humidity and temperature gradients are essentially evaporation gradients and optima are most conveniently expressed in terms of evaporation (Shelford '13*b*).

TABLE II.

EXPERIMENT 34. SHOWING THE REACTIONS OF *Phrynosoma* IN AN AIR TEMPERATURE GRADIENT.

The temperatures of the thirds of the cage are in each case indicated at the top of the column. Readings were taken at one-minute intervals. For discussion see page 105.

Minutes.	Experiment 34*a*. Temperature.			Experiment 34*b*. Temperature.			Experiment 34*c*.[1] Temperature.		
	29°.	33°.	38°.	38°.	33°.	29°.	37°.	29°.	27°.
1	3	3	4	3	4	3	3	2	5
2	4	2	4	3	5	2	3	2	5
3	4	2	4	3	6	1	3	2	5
4	4	2	4	4	4	2	3	4	3
5	3	2	5	4	4	2	4	4	2
6	3	2	5	4	5	1	4	3	3
7	3	4	3	4	5	1	4	3	3
8	3	4	3	4	5	1	4	3	3
9	3	4	3	4	5	1	5	3	2
10	3	4	3	4	5	1	5	3	2
11	3	4	3	4	5	1	5	3	2
12	4	3	3	4	5	1	4	5	1
13	2	4	4	4	5	1	4	5	1
14	2	4	4	4	4	2	4	6	0
15	2	4	4	3	5	2	4	5	1
16	2	2	4	3	5	2	4	5	1
17	2	4	4	3	5	2	4	5	0
18	2	3	5	4	5	1	5	5	0
19	2	3	5	4	5	1	6	4	0
20	2	3	5	5	4	1	6	4	1
21	2	3	5	5	4	1	6	4	0
22	1	5	4	5	4	1	6	4	0
23	1	5	4	4	5	1	7	3	0
24	1	5	4	4	5	1	7	3	0
25	1	5	4	3	6	1	7	3	0
26	1	4	5	3	6	1	7	3	0
27	1	4	5	3	6	1	6	3	1
28	1	4	5	3	6	1	6	3	1
29	1	4	5	3	6	1	6	4	0
30	1	4	5	6	3	1	6	4	0
31	1	4	5	6	3	1	6	4	0
32	2	2	6	5	4	1	6	4	0
33	1	2	7	5	4	1	6	4	0
34	1	4	5	5	4	1	6	4	0
35	1	4	5	6	3	1	5	5	0
36	1	4	5	5	4	1	5	4	1
37	1	4	5	5	4	1	5	4	1
38	1	4	5	5	4	1	5	4	1
39	1	4	5	6	3	1	4	5	1
40	1	4	5	5	4	1	4	5	1
	78 20%	144 36%	178 44%	167 42%	184 46%	49 12%	200 50%	153 38%	47 12%

[1] Substratum cooled to 20°.

3. *Substratum Temperature Gradient.*

The gradient cage was placed in a water bath so arranged that hot water flowed into the latter at one end and cold water at the other, the water being directed backward and forward beneath the cage so as to produce a gradient in the temperature of the substratum. This experiment was conducted with still air in the cage, *i. e.*, with air of approximately the same temperature as the substratum and consequently varying in evaporating power, and with air of a uniform temperature and evaporating power flowing across the gradient. The results of the two types of experiment checked very closely, indicating that the temperature of the substratum is the dominant controlling factor under these conditions. Both show an optimum substratum temperature of 35° to 40°. In general the animals which had been kept at the higher temperature, *i. e.*, in cage No. 2, oriented themselves in the gradient more quickly than those kept at the lower temperature (Experiments 26, 27 *a*, *b*, Table III.). When the animals gathered in the region of the optimum temperature an increase or decrease of the temperature of the gradient caused a corresponding movement of all the animals, which were repeatedly driven from end to end of the cage in this manner. The lizards often burrowed at or near the upper limit of the optimum region. They also often burrowed when the temperature was reduced as low as 20°.

When the animals were placed in a cage the substratum of which was gradually heated, rapid movements, interspersed with digging reactions began at 35°, and as the temperature approached 40°, all animals attempted to burrow. However, under experimental conditions, it was impossible to heat the substratum without having the lower layers of the soil at least as hot as the top, and the animals did not complete the burrowing reaction, but again moved about the cage rapidly. Above 40°, evidences of extreme discomfort, such as wide-open mouth, spasmodic movements, etc., were evidenced.

When animals in a sand-bottom cage were subjected to a continuous blast of air of increasing temperature from above, so as to heat the surface of the soil without greatly increasing the temperature of the lower layers of the soil, they became very

TABLE III.

SHOWING THE REACTIONS OF *Phrynosoma* IN A GRADIENT OF TEMPERATURE OF THE SUBSTRATUM.

Temperatures are indicated at the tops of columns. Readings were taken at intervals of one minute. In Experiment 27 the figures in italics indicate burrowing on the part of the number of individuals so designated. For discussion see page 107.

Minutes.	Experiment 26. Temperature.			Experiment 27. Temperature.		
	19°.	39°.	43°.	25°.	40°.	52°.
1	3	4	3	2	6	2
2	1	5	4	1 *1*	8	0
3	1	4	5	1 *1*	8	0
4	1	3	6	*1*	8	1
5	0	4	6	*1*	8	1
6	0	4	6	*1*	8	1
7	1	4	5	*1*	8	1
8	1	5	4	*1*	9	0
9	2	5	3	*1*	9	0
10	3	4	3	*1*	9	0
11	4	3	3	*1*	*1* 8	0
12	4	3	3	*1*	*1* 8	0
13	2	5	3	Redistributed		
14	1	5	4	21°	36°	46°
15	1	5	4	4	2	4
16	1	5	4	4	3	3
17	1	5	4	3	4	3
18	1	5	4	3	4	3
19	1	5	4	3	5	2
20	1	6	3	3	5	2
21	2	5	3	3	6	1
22	0	7	3	1	9	0
23	1	5	4	0	9 *1*	0
24	1	5	4	0	9 *1*	0
25	0	5	5	0	9 *1*	0
26	0	5	5			
27	0	5	5			
28	1	4	5			
29	0	5	5			
30	0	5	5			
31	0	5	5			
32	0	4	6			
33	0	5	5			
34	0	3	7			
35	0	4	6			
36	0	3	7			
37	0	3	7			
38	0	4	6			
39	1	3	6			
40	0	4	6			

restless as the temperature of the soil approached 40°. Most of the animals burrowed, or at least flattened their bodies close to the substratum as the surface temperature reached two or three

degrees above this. Unfortunately, in the apparatus available a higher air blast temperature than 43° could not be obtained.

The reaction to the temperature of the substratum, independent of the temperature and rate of movement of the air above and consequently independent of the evaporating power is the most definite and well defined response noted in the series, much more so than that in the air temperature and humidity experiments and must, therefore, represent an important factor in the daily and seasonal life of the animals. It is probable that this, and not the evaporating power of the air furnishes the most important direct stimulus determining the distribution and daily movements of the species. The surface of the soil in the Rio Grande valley region is cooler than that in the mesa habitat on account of its greater moisture content, and that in the mountains is cooler for this reason and also on account of the climatic variations due to higher altitude. The rise of temperature during the day stimulates the animal to burrow as a means of escaping the excessive heat. At least the surface temperature is of very great importance, as shown by the exceedingly consistent and regular behavior of the animals when this feature of their environment is varied. Unfortunately, but a single specimen of *P. douglasii* was available at the time the preceding experiments were conducted, and this one was taken at the same place as those of the more abundant species, *i. e.*, on the mesa. It would be of great profit to repeat this experiment with individuals of various species taken in entirely different habitats.

III. Conclusions.

1. The optimum evaporation rate of air for *Phrynosoma modestum* lies between 1.5 and 3 c.c. per hour, as measured by a standard atmometer. This is approximately the average evaporation rate of outdoor air in the natural habitat of the species, in sunlight, with a light breeze and a temperature of 30°. The reaction of the animals to a humidity gradient is not definite unless the gradient be a steep one. Daily variation in the habitat of the species is rather large.

2. The reaction to an air temperature gradient is more definite than the preceding. As temperature varies, the evaporating

power of the air also varies, and thus we get an evaporation gradient plus a temperature gradient, so that the gradient under these conditions is a double one. The optimum temperature is in the neighborhood of 30° when the humidity and rate of flow of the air is such as to produce an evaporation of 3 c.c. per hour, as measured by a standard atmometer.

3. Air humidity, air temperature and air current gradients are all essentially evaporation gradients, and the optimum in each case is a function of the evaporating power of the air.

4. The most definite and clear cut reaction in a gradient shown in this series of experiments is that in the substratum temperature gradient. This reaction is, to a great degree, at least, independent of accompanying differences in air temperature and humidity, or at least, the effect of these variations is overshadowed by the response to the gradient in substratum temperature. The optimum substratum temperature is rather definite—between 36° and 40°, and at the upper limit a very definite reaction (burrowing) takes place. This optimum is correlated with the other optima given above, in that the substratum temperature, under the conditions given in paragraph 1, is usually from five to ten degrees above the temperature of the air. Soil temperature (surface) is evidently of very great importance in determining details of seasonal and daily life, having as well its accompanying effect on the distribution of the species.

5. It is dangerous to ascribe to any one factor or group of factors the supreme rôle in determining the seasonal or general distribution of a species. The factors are certainly not the same for all species even in the same environment and before definite conclusions can be drawn a careful analysis of the habitat must be made, and experimental data must be obtained as to the reaction of the animals in gradients involving the factors capable of variation.

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CHART I. Showing the reactions of *Phrynosoma* in gradients of the evaporating power of air. In the experiments illustrated this gradient was established by varying the humidity of the air with which the animals came into contact. The manner in which the gradient was established and the records made was as follows: A rectangular cage approximately 50 cm. × 10 cm. and 5 cm. deep was so arranged that currents of air from different sources could be directed across different sections of the cage. The openings were so arranged that the air passing across a given third of the cage was of uniform character throughout the experimental period, while that in each third differed as to humidity, but in no other way from that in the other sections of the cage. The animal could pass freely from one part of the cage to another. See text for more complete description of the apparatus. In the chart, each section between the numbered scales represents the record of a twenty-minute experiment, the distance between the scales representing the length of the cage, and the vertical length of the chart the time, twenty minutes, each division on the scale representing ten seconds. The curve in this space represents the movements of the animal under observation, and as the time-component is vertical and the space-component is horizontal, the parts of the curve more nearly horizontal represent the most rapid movements, while the vertical parts of the curve indicate that the animal was at rest during the length of time indicated on the scale. Thus, in chart 2*a* (the fourth from the left), the animal, introduced near the centre of the cage, remains in that position for approximately one minute, and then moves toward the left end of the cage, the movement occupying a period of ten seconds. The lizard remains in this position for a little over twenty seconds, and then moves toward the other end of the cage, where he arrives at the end of the second minute. Here he remains for three minutes, when he again moves toward the left end of the cage.

The figures accompanied by letters at the top of each section of the chart designate the number of the experiment. Experiments designated by the same number and consecutive letters were performed with the same animal and consecutively. Otherwise the experiment numbers are entirely arbitrary. The upper line of three figures in each section *e. g.*, 34, 19, 4, in 1*a*, indicates the relative humidity of the air passing through the corresponding thirds of the cage, as computed from standard wet and dry bulb thermometer readings. *S S S* in 1*c* and 5*c* indicates that in these control experiments the air was still and the same in all sections of the cage. The lower line of figures, *e. g.*, .5, .9, 1.5 in 1*a*, represents the standard atmometer reading, for a similar twenty-minute period, of the air passing through the corresponding third of the cage. Thus, in Experiment 1*a*, the relative humidity of the air passing through the left-hand third of the cage was 34 per cent., and the corresponding atmometer reading .5 c.c. In the middle third the relative humidity was 19 per cent. and the atmometer reading .9 c.c., while in the right-hand third the relative humidity was 4 per cent. and the atmometer reading correspondingly high, or 1.5 c.c. The rate at which the air passed across the cage was in each case 25 liters per sec.

Where the record is in the form of a dotted line, it indicates that the animal, in the corresponding period, either attempted to escape from the cage or to burrow in the sand on the cage bottom. In the records of experiments 3 and 4*a* the circles and the parallel lines following indicate that the animal burrowed under the sand and remained covered for the period indicated. In the record of experiment 5*a* the circle indicates that the animal was under the surface of the sand for a brief time only. For discussion of individual experiments see text.

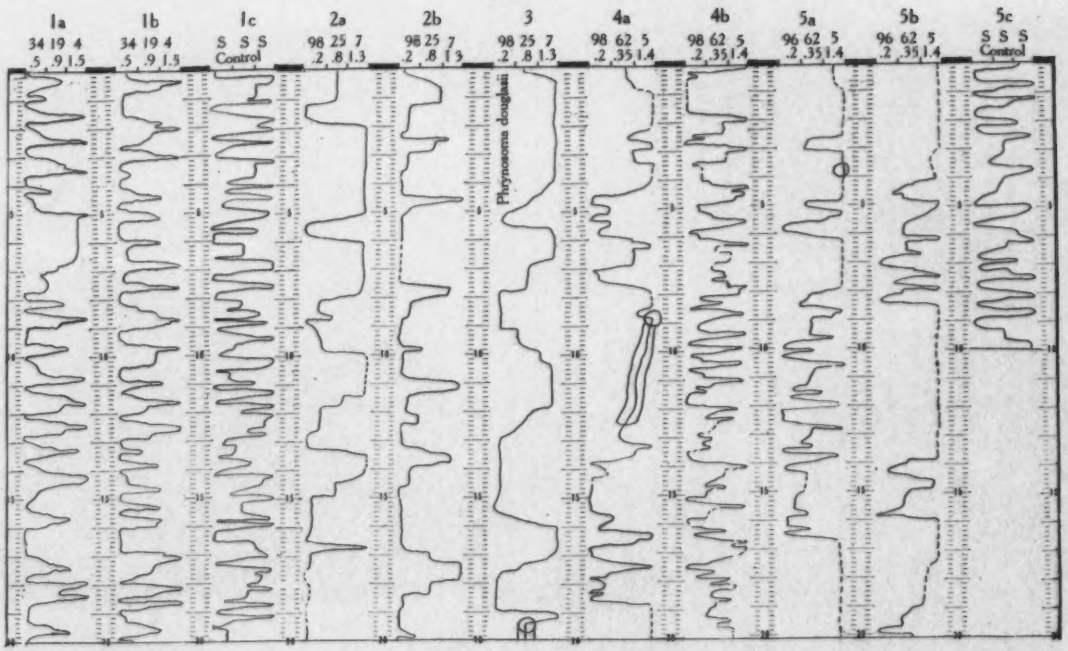
Experiments 1a, 1b, Control, 1c. (*Chart I*).—The tracing which is the graphic record of experiment 1*a* shows that the animal, for the first minute and a half, remained in the two portions of the cage where evaporation was lowest, then, at intervals about one minute made brief visits to the drier section. Following the fifth minute there is recorded a stay of over one minute in the driest third of the cage. Aside from this all the longer rest periods were spent in the opposite end of the cage. It will be noticed that, in general, the apices of the curves at the right are sharper than those at the left, indicating quicker turning in this region. In the first minute the animal turns away from the drier portion of the cage twice, once in the fifth minute, again in the eighth, tenth, sixteenth, eighteenth and twentieth minutes. There is only one turning back from the more moist regions, in the nineteenth minute.

The record of 1*b* is of almost the same character, with more avoidance of the dry end, as there are ten turnings during this twenty-minute period.

The control, 1*c*, in which the same animal was used shows somewhat greater activity than the two previous experiments, as the line crosses the area representing the cage a greater number of times. This curve, however, is not in the least one-sided, showing that the animal avoided no region of the cage. As indicated at the top of the chart, there was no air current through the cage in the control.

Experiments 2a, 2b.—In this experiment one end of the cage was supplied with air nearly "saturated" with moisture, as is indicated by the relative humidity of 98 per cent. and an atmometer reading of .2 c.c. During the first twenty-minute period, represented by tracing 2*a* the animal spent a littlemore time in the dry end than in the very moist region. During the second twenty-minute period (2*b*) the animal reached the dry third but six times, turning back almost immediately in

CHART I


1a
34 19 4
.5 .9 1.5
1b
34 19 4
.5 .9 1.5
1c
S S S
Control
2a
98 25 7
.2 .8 1.3
2b
98 25 7
.2 .8 1.3
3
98 25 7
.2 .8 1.3
Physocoma douglasii
4a
98 62 5
.2 .35 1.4
4b
98 62 5
.2 .35 1.4
5a
96 62 5
.2 .35 1.4
5b
96 62 5
.2 .35 1.4
5c
S S S
Control

each case. The longest stay in the dry end was in the eighteenth minute—about forty seconds.

Experiment 3.—This experiment is typical of those performed with *Phrynosoma douglasii*, and shows very little. The animal was inclined to be sluggish and finally, at the end of the period, burrowed in the middle section of the cage, where the humidity was 25 per cent. and the evaporation .8 c.c.

Experiments 4a, 4b.—This animal, beginning in the center section of the cage, moved, after nearly one minute, to the dry end, where attempts to escape, indicated by the dotted line, were recorded. It turned back from the medium section twice in the next two minutes, attempting to escape during the second stay in the dry section, and then spent nearly two minutes in the wet end, turning back twice from the medium air. A short visit to the dry end was then recorded, and then, at the beginning of the ninth minute, a marked escape reaction in the dry end, followed by a period of three and one half minutes, when the animal burrowed beneath the soil. While under the soil, the animal gradually moved toward the center of the cage and finally emerged there. Thereafter visits to the dry third were of very short duration until near the end of the period when escape at this end is again attempted. During the second period (*4b*) the animal was very active, and, except that, in general, the apices of the curves in the section of the diagram representing the dry end of the cage are more acute, indicating quicker turning here, very little can be said. There were many escape reactions in all parts of the cage.

Experiment 5a, 5b, Control 5c.—This experiment shows more definitely than any other the relation between stimulation to escape attempts and the length of stay in the dry air. It will be noted that escape attempts were recorded throughout each stay of any length in the dry air, and that, aside from these periods of stimulation the animal spent the greater part of the time in the center section of the cage. One short burrowing movement was recorded at the end of the fourth minute of the first period. The control, as elsewhere, showed a rather active movement from end to end of the cage, but no avoidance of any region.